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FINAL VACCINE PRODUCTION REPORT

**Mali Livestock Development Project
Contract No. 688-0218-C-00-5057-00**

Prepared for:

**USAID/Mali
and the
Government of the Republic of Mali**

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SUMMARY

The following is a brief summary of recommendations:

- (1) Electricity: Install capacitor; raise peak load limit to 250 kW.
- (2) Water: No action appears necessary; new water system delivering good water.
- (3) Steam: Investigate use of adjacent electric powered steam generators in lieu of centrally located oil-fired generators. Give steam generator replacement second priority.
- (4) Pure Water for Vaccine Production: Use electric powered electric still for primary purification; go to RO system for additional purification for media preparation and other more fastidious needs.
- (5) Funding: Try to return to pre-1988 system of central bank account rather than present direct Treasury accounting.
- (6) Freeze-Dryers: Weigh advantages of larger Series 251 Virtis dryer with added capital outlay vs. smaller Series 101 dryer requiring more dryer runs, increased testing costs, etc. Give freeze-dryer procurement top priority.
- (7) Spare Parts: Order if resources permit. Give spare parts procurement perhaps third priority.
- (8) Production Techniques: Use safety cabinets for critical steps having sterility concerns. Investigate Plum Island heat-stable rinderpest vaccine versus Yilma recombinant vaccine. Give serious consideration to adopting one of these products as new protocol.
- (9) Packaging: Pursue using plastic bottles procured in Bamako as containers for inactivated products. Investigate feasibility of combination products using inactivated products as diluents.
- (10) Storage: Lab storage seems adequate. Purchase second cooler in Bamako for storage of tested product so tested and untested products are stored in separate coolers. Put emphasis on proper storage of product in the "cold chain".
- (11) Sale and Distribution: Assign person with authority to actively pursue marketing opportunities. This might include development of new products, new product combinations, markets in adjacent countries, improving distribution techniques by DNE, etc. If products are useful and innovative, higher prices (above cost) may be charged.

(12) Quality Control: Routinely assay samples from the field as well as in-house for potency to insure quality at end-user level. Also assay products at end of dating to insure expiration date is consistent with true shelf-life. Vacuum test dried product both out of the dryer and throughout shelf-life. Utilize ELISA testing of serum samples as excellent indicator of rinderpest vaccination effectiveness. Give moisture testing of product low priority.

(13) Training: In operations, training in marketing techniques coupled with efficient cost accounting in order to work toward more self-sufficiency in vaccine production. Include periodic trips to key labs in Europe and the U. S. to try to locate new products of use in West Africa. In maintenance and installation, see if visits to Mali by freeze-dryer, sterilizer, and steam generator company service engineers might give as much benefit as Malian trainee visits to U.S. plants.

(14) Miscellaneous: Peace Corps Poultry Project - cooperate with volunteers on hatching healthy immunized chicks for village projects; work closely to monitor disease problems in these birds as sentinels of problems in general poultry population. Mineral Blocks - This is a more complex problem than it first appears. Use of high pressure presses to form blocks as is done in the U.S. does not seem practical for Mali. Formulations with high phosphate content are hard to press and are too granular for palatability by cattle. Investigate use of "candy blocks" with high molasses content?

SCOPE OF WORK

The following is the Scope of Work approved by the Mission and Ministry for the technical study of vaccine production:

OBJECTIVES

1. Assist the Central Veterinary Laboratory (CVL) in improving the present technical procedures being used for the production of vaccines.
2. Identify and make recommendations for resolving production constraints related to organizational management, procurement, and inventory of reagents and biologicals, and technical procedures taking into consideration economic realities.

TERMS OF REFERENCE

- I. Undertake a detailed technical analysis of the entire vaccine production process.
- II. Assess and make recommendations to increase the efficiency of production in terms of procurement of basic materials; utilization of equipment; techniques of production and packaging; systems of storage; and procedures for sale and distribution.
- III. Assess and make recommendations for improving the efficacy of vaccine control procedures.
- IV. Assess and make recommendations to improve the packaging of vaccine to insure their quality and better respond to marketing needs.
- V. Based on technical findings, make an analysis of additional training needs in the area of operation, maintenance, and installation of equipment.
- VI. Prepare a final report detailing your findings taking into consideration the objectives stated above. Include precise recommendations relating to the entire vaccine production process. Include a complete analysis of equipment needs with recommendations for replacement of existing equipment. Prepare technical specifications for all proposed equipment in sufficient detail so purchase orders may be prepared. Prepare detailed descriptions of training needed. Include where possible the names of institutions where recommended additional training can be obtained.

I. TECHNICAL ANALYSIS OF VACCINE PRODUCTION PROCESS

The Central Veterinary Laboratory (CVL) is the sole veterinary vaccine production facility in the Republic of Mali and is the largest in West Africa. As originally designed in 1970, it has the capacity to produce sufficient vaccine for the majority of needs for this part of Africa. Realities that have ensued in the twenty years since the laboratory was built have hampered efforts to utilize its capabilities to their fullest extent. As is true of any facility, constant use of equipment and buildings over a twenty year period has resulted in the need for replacement and improvement of key parts of the operation.

Vaccine Production Division

The Vaccine Production Division has five sections: 1) Rinderpest Vaccine Production; 2) Pleuropneumonia Vaccine Production; 3) Bacterial Products Production 4) Poultry Products and 5) Internal Quality Control.

It was not possible to observe actual production operations during the time of this visit because the steam line supplying the production unit was ruptured and was in the process of being repaired. This, in turn, made the autoclaves inoperative. In place of observations of actual work in progress, observations were made of the facilities, copies of the protocols of production were provided and analyses made from them as requested in the Terms of Reference Item I. Copies of the protocols (in French) are included in Appendix A.

II. RECOMMENDATIONS TO INCREASE PRODUCTION EFFICIENCY

A. PROCUREMENT OF BASIC MATERIALS

Laboratory personnel have used splendid ingenuity in their use of locally obtainable materials and in the reuse of imported materials.

Electricity

There is one over-riding concern that hovers over all considerations of ways to increase production efficiency... The cost of electricity in Bamako--among the most expensive in the world-- is approximately 59 CFA per kilowatt hour or in the neighborhood of \$0.17 at current exchange rates. This consideration must be weighed in all recommendations.

The laboratory has a reliable standby generator which switches on automatically when municipal power fails. Use of diesel backup power, however, is even more expensive.

During a recent 24 hour period when backup power was used, the cost was approximately 350,000 CFA (about \$1000) or over \$40 per hour.

The laboratory has had prepared a proposal from at least two contractors in Bamako to install a capacitor with ancillary equipment to correct the power factor problem they have during periods of peak power use. I discussed this problem with Mr. Robert Smith of the Bechtel Engineering Group in San Francisco (415-768-2892). He confirmed that this is a common problem in situations such as that at the lab and he says that the proposals seem to be well designed. There are advantages to having the variable solution (Solution II) due to the wide variation in power demand during the production year.

RECOMMENDATION: Due to the fact that the lab can recoup its initial outlay in 9-14 months, it makes very good sense to get the power quota raised to 250KW and to install the necessary "batterie de condensateur". Because the contractors have bid at not more than \$12,000 for installing this using locally available equipment, it does not seem expeditious to think about overseas procurement of the equipment.

Water

This is an area where needs have been met very effectively. The CVL has recently completed a reserve water system that draws directly from the Niger River. The system was designed by a Malian architect and includes a pumping station, water treatment plant and an underground reservoir located at the animal holding facility with supply lines to a storage tower on the laboratory grounds. The system is capable of supplying 30 cubic meters (about 1000 cubic feet) of treated water to the lab per hour.

RECOMMENDATION: The CVL seems to be very adequately supplied with sufficient water at sufficient pressure to meet all reasonable needs such as shutdown of municipal supply or low availability during times of drought. Samples of treated river water were submitted for analysis. See discussion under "Pure Water for Vaccine Production".

Steam

Steam is presently generated in a central plant and piped where needed throughout the laboratory compound in insulated underground pipes. The generators are old and all units need replacement. In addition, the pipelines are corroded thus they weaken and rupture and leak. The need for steam has changed both in quantity and in number of needed locations over the years and it now appears needed

only to supply three Amsco autoclaves in the Central Service area.

RECOMMENDATION: Please see further discussion in UTILIZATION OF EQUIPMENT Section

Pure Water for Vaccine Production

Selection of proper equipment has to take into consideration two factors: 1) the high cost of electricity, and 2) the high cost of obtaining cartridge refills for equipment using ion exchange, reverse osmosis, membrane filtration, etc., considering their efficiency and useful life.

Samples of water were obtained from the Bamako municipal supply, the alternate river source recently brought on line by CVL and from the still supplying pure water to the laboratory. A sample of the water from the CVL water treatment plant was submitted to the free analysis service offered by Barnstead/Thermolyne for recommendations. The people conducting the analysis were favorably impressed with the quality of the water. A copy of the analysis may be found on page 42D1 (Appendix M).

RECOMMENDATION: Emphasis should be placed on the special conditions existing in Bamako, i.e. the cost of electricity versus the cost of imported cartridges. Selection of the best system for the lab should be based upon the recommendations on information gained from the water analysis. Also see discussion under UTILIZATION OF EQUIPMENT.

Operational Funding

Perhaps operational funds are not ordinarily considered a basic material but the method by which funds are obtained has a profound effect on the efficiency of operation of the laboratory. I am told funding of enterprises in Mali falls into three general categories: 1) fully funded by the government; 2) independent of government support; and 3) receiving partial government support through subsidy. CVL is in the third category. Prior to 1988, revenue from sales of vaccine was deposited into a CVL bank account in the Central Bank and CVL could draw on that account. In 1987, this method was changed to placement of revenue into the Government of Mali General Fund with payment of subsidies from government budget accounts. CVL still has funds for which it was eligible in January 1988 that have not been received. If CVL could return to the pre-1987 method of having access to funds in an account in the Central Bank, it would seem that flexibility in responding to urgent needs would be much enhanced.

RECOMMENDATION: Efforts should be made at the Mission level and at the Ministry level to re-establish the pre-1988 method. If this is not re-established, it would appear that efforts to make the lab more semi-autonomous with abilities to expand sales both within and outside the country will be slowed and made cumbersome.

B. UTILIZATION OF EQUIPMENT

Twenty years of use is showing its effects in the utilization of equipment:

Freeze-Dryers

The two smaller REPP dryers purchased in 1963 have exceeded their useful life and should be taken out of service. Some parts for them are no longer available. They are also too small for economic operation.

RECOMMENDATION: There appear to be two options that need investigating: 1) replace the two smaller 41 series dryers with one new 101 series dryer to complement the present 101 series dryer, or 2) replace the two smaller dryers with one new larger dryer (251 series) with increased capacity plus the present 101. The reason that CVL people would like to consider option 2 is that it would allow much larger serial lot sizes to be produced that, in turn, would allow the present costs of testing multiple small serials to be significantly reduced. This would also simplify the logistics of producing needed doses because fewer dryer runs would be involved. The disadvantage of the larger unit would be the capital costs would be significantly larger. Various sizes and makes of dryers were considered. There was consensus that purchase of another Virtis was the best choice because of the longtime familiarity with this make and the possibilities for commonality of parts. The need for automatic stoppering that can accommodate different size vials in the same dryer run was also recognized.

Specifications for both new dryer alternatives are shown in Appendix B.

Steam Generators

The steam generation capacity originally provided in 1970 was one Clayton 30 HP unit and two Clayton 75 HP units. These units are oil fired and were designed to provide sufficient steam to operate the Amsco autoclaves, operate the Ultra-Pure Water Still and provide heat for maintenance of stable temperatures in the Production Building. At full capacity these units were originally rated at over 6 million BTUs per hour. At the present time, the units are beyond their useful life and due to the inefficiencies of operation (leaks in the supply lines and

in the deteriorated insulation), the units are run only about four hours per week when all autoclaving is scheduled and distilled water produced. The units are now so inefficient that threshold pressure is never attained and they run constantly throughout the four hours. During this short run, oil consumption is about 60 liters per hour. The present cost of fuel oil in Bamako is about 210 CFA/liter (more than \$0.60/liter). Total cost for this four-hour run is therefore over 150,000 CFA (about \$150).

It appears the need for steam has significantly changed since 1970. At present, only sufficient steam is needed to operate the autoclaves and perhaps produce pure water in the Production Building although it appears that an electrically powered still would be more economic than a steam-powered one.

The boiler feed water treatment equipment is no longer functional and feed water is used directly from the lab supply.

As much as possible of the present steam supply lines were inspected. The area around the manhole in the front of the Production Building had been torn up prior to the inspection in order to make repairs to a corroded section of pipe that was leaking near the building entrance. This work thus exposed a usually inaccessible area. Insulation in the supply line was deteriorated and both the supply and return pipes were corroded at this location. The supply line near to one autoclave was also inspected above the autoclave as it entered from the false ceiling. The same conditions were seen here. Section personnel related an incident in which the steam line ruptured in this location and sent live steam cascading into the work area forcing evacuation of the area. Insulation on supply lines near the autoclave itself was seen to be pulled away with uninsulated pipe exposed. Replacement of the oil-fired generators and the steam supply lines and returns is deemed to be a costly project.

An in-depth discussion was carried out over whether it would be more practical to locate electrically powered steam generators near the autoclaves or to replace the present oil-fired units with new more efficient units from Clayton. The water analysis disclosed that the treated Niger River water had only 17.5 ppm of calcium. No analysis for silica was available although it appeared from the other portions of the analysis that the silica content would not exceed 20 ppm, the threshold value for Clayton steam generators.

RECOMMENDATION: It seems that it is necessary to assess both of the options available: 1) replacement of the present oil-fired generators with new oil-fired generators and new steam supply lines, and 2) purchase electric powered

point-of-use steam generators that supply the needs of individual sterilizers and abandon the present central steam plant system of operation. Each has advantages. By adopting the oil-fired generator approach, less new construction would be involved, and existing lines could be upgraded by the maintenance section as needs arise. By adopting the electric generator approach, individual autoclaves could be activated more on an as needed basis rather than having to concentrate all autoclaving activity into a relatively short period to accommodate the costly constraints of the present central system. This approach appears to be justifiable on a cost as well as on an efficiency basis. Large electric units ($2 \times 60 \text{ kW} \times 4 \text{ hours} \times 59 \text{ CFA/kWhr}$) would cost 35,400 CFA while 4 hours under an oil-fired system would cost 63,000 CFA as determined above (based on two units using 37.5 lph/unit). There is need to purchase sufficient numbers of spare heating elements and other spare parts for the electrical generators, however, to insure long term operation because of the tendency of the electric heating elements to fail sooner than with the oil-fired system. Specifications for both approaches are shown in Appendix C.

Autoclaves

During in-depth discussions with Production people, it was determined that two gravity single door autoclaves with the dimensions of 24 x 36 x 60 rather than the three with these dimensions presently installed would adequately serve needs in the Production area. Inspection of the presently installed units revealed the effects of 20 years of use. Insulation is torn, unit controls are not fully functional, and the units in general reflect years of use.

RECOMMENDATION: Purchase two new 24 x 36 x 60 gravity single door units with needed piping, valves, traps, etc.. If use of electrically powered steam generators seems more feasible, thoroughly discuss renovating the present sites of installation so that steam generators could be placed adjacent to the autoclaves or perhaps be mounted relatively nearby (within 16 meters) so that supply and drain pipes would be short and efficiency would be much easier to maintain. Note should be taken that the present units are functional and could be used for the near future if other priorities mandated postponement of replacement. Specifications for the proposed autoclaves are shown in Appendix D.

Water Stills

Quality and characteristics of the feed water have an important effect on the type of still that will best fulfill the needs for vaccine production. There is some indication from comments that were made that present water quality is

having an adverse effect on cell growth for rinderpest vaccine production and perhaps on other products. The various sections were polled to determine what their best estimates of quality water needs were. Estimates are as follows:

| | |
|----------------------|------------------------|
| rinderpest..... | 250-300 liters/month |
| pleuropneumonia..... | 500 liters/month |
| poultry..... | 300 liters/month |
| killed vaccines..... | 2000-3000 liters/month |

estimated total needs per month..4050-4100 liters/month

Discussions also indicated that the Production Unit on occasion provides pure water to public health personnel, the telephone company, and other parts of the lab. These people reciprocate with favors in return. Consumption is not of high volume. It seems prudent to insure there is some excess capacity to meet unexpected increases in use.

Another factor that must be considered in selecting a system is the rapidity with which prefilters clog and distillation vessels show excessive residue buildup at present. The analysis conducted by Barnstead/Thermolyne indicate that the CVL treated water is of unexpectedly good quality with low calcium content. It is not entirely clear why cartridges plug up so rapidly.

RECOMMENDATION: Based upon recommendations from the Barnstead/Thermolyne analysis, CVL water should be first purified by running it through a classic still. A still with 38 lpi. capacity would meet peak production needs when running at roughly four hours per day. The water appears soft enough that pretreatment would not be a major concern. This should make available water that will meet general purpose needs. If problems persist, water can be further purified through the Millipore RO system that is in place or with a system that provides comparable purity. This may be required for tissue culture media and elsewhere where pyrogens are a concern. Use of reverse osmosis directly from tap water seems to have met with problems when used in other sections of the lab because replacement cartridges are expensive and hard to obtain and prefilters plug up as rapidly as every 3 to 4 days. The reasons for this are not readily apparent based upon the water analysis. Perhaps running the water through a common household water filter would be an easy solution to the plugging of prefilter cartridges. Such units are available in California hardware stores for about \$35.00 with cartridge refills at about \$4.00 each. Specifications for a classic still with 38 lph capacity are shown in Appendix E.

Other equipment

During the tour of facilities, various pieces of equipment were seen that were not fully operational. These pieces of equipment fall into three general categories: 1) in working order but spare parts were needed for continued maintenance, 2) non-functioning because replacement parts were needed but were not available, and 3) the equipment was generally operational but not economically feasible to use. Equipment has been categorized as follows:

In Working Order

Virtis sharp freezer
Industrial Voltage Regulator
Automatic Power Transfer Switch
Consolidated Sterilizers (change to 380V/3 phase)

Needing replacement parts

Ingersoll-Rand air compressor (needs filters)
Dunham-Bush package chillers
Production Building Incinerator (needs new motor)

Not economically viable

Cryogenics Technology liquid nitrogen generator
Hytek demineralizer (?)
Hytek deionizer (?)

RECOMMENDATION: Procure the spare parts shown on the Director's list that has been edited and included in this report as Appendix F.

C. TECHNIQUES OF PRODUCTION

As stated previously, it was not possible to observe actual production because of the lack of steam to the autoclaves in the Central Service area. The following is based upon review of production protocols and walk around observations.

Work requiring sterile conditions is carried out in an arrangement of locally constructed hoods that are glass panels mounted in wooden frames. These hoods appear to have been part of the original construction. No use of safety cabinets with HEPA filtered air was seen although key labs in the research and new microbiology wing had them. From discussions, it would appear that sterility is sometimes a problem in the Production areas.

RECOMMENDATION: Critical operations such as preparation of kidneys for trypsinization, preparation of cell cultures, inoculation of virus, changes of media, harvest of infected fluids, vial filling, media preparation, culture expansions, etc. are done more cleanly if done in a safety cabinet. Procurement of several cabinets for at least rinderpest, pleuropneumonia, and poultry should be investigated. The Baker and NuAire hoods seen in other parts of the laboratory are excellent.

Dr. Charles Mebus at the Plum Island Animal Disease Lab working in cooperation with the veterinary lab in Niger has been developing a more heat stable rinderpest vaccine using the Plowright strain of virus grown in Vero cells. This vaccine has a superior stabilizer and is dried using a superior combination of shelf temperature and bleeding of air into the dryer chamber to produce a vaccine that is capable of holding immunizing capabilities after 200 days at 37 C. This stability makes possible the elimination of the cold chain in transporting and using vaccine in the field. A copy of a preprint of a paper prepared describing this vaccine may be found on page 42E1 (Appendix N).

RECOMMENDATION: Gain more information about the Plum Island vaccine and compare it with the product that Dr. T. Yilma of the University of California at Davis has developed. Dr. Yilma has developed a recombinant vaccine using vaccinia as a vector. Duration of immunity studies have not been done with this product. Various authorities including those at CVL are exploring the possible safety factors involved with the widespread introduction of vaccinia into ruminants and whether this could be a safety problem if immunodepressed humans should be exposed to the virus. The inherent heat stability and the possibility of teaching herdsmen to do their own vaccination against rinderpest offers some interesting possibilities for increasing vaccination percentages using Dr. Yilma's vaccine. CVL's position of careful and deliberate review of the attributes of this innovative product is a good one, in my opinion.

CVL should continue to evaluate alternative production protocols in light of advances in production techniques.

From limited discussions I have had, it would appear that the period of greatest demand for vaccine in Mali is about the same as it is in the United States, roughly November through February. If I understood correctly, so-called "dating" or the period stated on the label as during which dried vaccine maintains at least minimum titer can be as long as 3 years. This may be overly long and studies should be initiated to substantiate that the products are, in fact, that stable (see QUALITY CONTROL discussion).

RECOMMENDATION; Buildup of inventory be scheduled for the months before greatest demand so it is not necessary to have either back orders or force use of significant amounts of vaccine that have been held over from the previous season. This also allows lab resources such as steam supply and pure water production to be concentrated into as short a period of time as possible in order to reduce costs as much as possible. There should not be undue concern if production facilities remain idle for periods during the year. This is not uncommon in labs in the United States also, especially at those with seasonal product lines. The Ministry should make funds available enough prior to heavy production so needed materials and equipment are ready to go.

D. METHODS OF PACKAGING

In the specifications for the replacement lyophilizer, it is noted that the buyer (CVL) agrees to provide samples of all bottles used. At present, the size of bottle that is being used does not fit the dryer trays and results in undue effort to load the bottles in the trays.

The design of the stopper being used is from France. A design that is available in the U.S. has a large single slot. It would appear that the U.S. design allows for more efficient exhaust of moisture and should thus bring about a minor improvement in drying efficiency.

RECOMMENDATION: Try a few dryer runs using the U.S. design and see if there is any noticeable improvement and, if so, whether the cost and ease of procurement justifies changing the stopper design used.

Also see discussion under IV. ASSESSMENT AND RECOMMENDATIONS FOR IMPROVING PACKAGING OF VACCINES

E. METHODS OF STORAGE

Storage of product at CVL seems to be well managed. The refrigeration units for the vaccine storage area have recently been overhauled and the temperature in the holding cooler was well within acceptable limits. However, there is no separation of untested and satisfactorily tested vaccine lots and there is no redundancy in cooler space in case one of the units fails.

RECOMMENDATION: A second cooler for storage of satisfactorily tested product be set up separately from the untested product. In emergencies, these stocks could be carefully stored apart in the functioning cooler. Procurement of this equipment is better done through local purchase in Mali rather than through third country procurement.

RECOMMENDATION: Emphasis be placed on reviewing the adequacy of the cold chain after products leave the laboratory. A very useful method to do this, in my opinion, would be to start with a thorough review of the results of the ELISA testing of rinderpest vaccinated cattle that the lab is presently conducting. If this survey indicates cattle are not being immunized, a series of samples should be obtained along the cold chain to try to find the weak links. Please see further discussion under IV. QUALITY CONTROL PROCEDURES.

F. PROCEDURES FOR SALE AND DISTRIBUTIONS

This is an area of consideration that seems to have many interesting possibilities. In the United States, this is an area of intense activity in licensed veterinary biological laboratories. Many labs have large staffs of sales people who are hired to make regular personal visits on users and potential users of vaccines to closely monitor their needs and, of course, attempt to get them to buy the line of products. At the laboratory itself, there are usually other staff members who gather information on "market surveys" and activities of competitors. In the U. S., many of the "new products" that come out of research are the result of discussions sales people have with the Research and Development people. It is an axiom in the veterinary vaccine business that money is made by introducing as many new and unique products that fill a "niche" as possible. Laboratories that are most successful in introducing new products remain among the most successful year after year. "Me-Too" products produce only moderate return and usually lag far behind in market share. Top people in marketing in the U. S. are extremely valuable to their companies based on their acumen in finding consumer wants, working with "bench" people to make an attractive product and then getting the attention of endusers to the value of the new products.

RECOMMENDATION: The Director of CVL appoint a person with responsibilities for marketing. It is noted that the situation in Mali is far from the highly competitive environment that exists in the United States but there appear to be opportunities. This person would probably be more correctly be categorized in the Malian situation as being a lobbyist rather than a pure sales person in that he will be working largely with other agencies involved in vaccine programs. However, this person could, for example, work closely with diagnostic people at all levels in Bamako and in the field and in other West African countries to detect needs for new products and with research people to see if these needed products can be developed. He (or she)

would also monitor how well the vaccines were serving the endusers and focus on those areas of distribution or actual field vaccination procedures that were perhaps deficient using his position of authority to get changes made. The scope of his responsibilities would not be confined to Mali. Marketing people are always watching the competition. There is competition in West Africa. Malian vaccine could be used in neighboring countries. Again, is Mali competing with "me-too" products or can CVL come up with innovative products that will better fill the needs both in Mali and in the surrounding areas? It is felt that there may be a small but real market for innovative quality products that can be sold for substantially more than prevailing prices of neighboring government subsidized labs. Here again the key is the acumen of the people involved with new product development. An idea ill-conceived is a failure; an idea that is right can create significant revenue and give the laboratory the means to grow and work toward self-sufficiency.

A lab in the U.S. that has consistently remained high in sales and consumer acceptance of their products has had a regular program of visiting key laboratories in Europe each year. They develop a rapport with key scientists throughout the world. As a consequence they have been many times forerunners in finding original research that could be developed into new and unique products. It seems to me the opportunity for a marketing person at CVL to begin to look into possibilities such as this offers a very viable way to increase the revenue of CVL. For instance, at the present time it appears that Mauritania is hosting a field trial in September of a new Rift Valley fever vaccine that was developed at the Plum Island lab. There is also work going on at Plum Island in developing an improved sheep pox vaccine.

The Director of CVL should appoint a person as described above with responsibilities as outlined.

III. ASSESSMENT AND RECOMMENDATIONS FOR IMPROVING QUALITY CONTROL PROCEDURES

Rinderpest vaccine is regulated by requirements of the Pan African Rinderpest Control (PARC) group. The minimum titer allowed is $10^{2.5}$ /dose. It appears that adequate titers are maintained in vaccine inventories held in CVL coolers. The critical question is what system is in place to monitor the titers of vaccine at the point of use?

RECOMMENDATION: In cooperation with DNE, it is suggested that several times during the vaccination campaigns randomly selected bottles be returned to the lab for titering. Preferably make the selection from multiple lots of vaccine. Based on the results of this study, focus on areas of weakness, if any, in the cold chain.

Alternately, analyze the results of ELISA testing of serum samples that is now in progress at CVL. If adequate seroconversions are not being seen, begin investigations into where the cold chain can be strengthened.

It is not clear to me whether the expiration date placed on the label of each bottle is the result of validation by end of dating studies or has been arbitrarily selected under PARC guidelines.

RECOMMENDATION: If the latter is the case, it would be useful to hold retention samples for titering at regular intervals up to and beyond the selected expiration date to verify that the dating interval selected reasonably reflects the true shelf life of the product.

The laboratory has requested specifications for equipment to vacuum test bottles as they come out of the freeze-dryer. Specifications for a vacuum tester is included in Appendix H.

RECOMMENDATION: Vacuum test each lot as it emerges from the dryer. In addition, conduct routine vacuum testing on all bottles returned from the field, held as retention samples, and selected samples prior to shipment to validate that vacuum seen immediately after drying is being maintained throughout the shelf life.

The laboratory has requested specifications for equipment to test dried vaccine samples for moisture content. Setting a maximum allowable moisture content is more complex than it would first appear. The stabilizer selected will have an effect on the moisture content. Stabilizers that contain sucrose or gelatin can be expected to maintain higher moisture levels without deleterious effects. Specifications for equipment needed to run an oven-dry moisture test is included in Appendix I. Protocol for conducting the oven-dry method is included in Appendix J.

RECOMMENDATION: Conduct moisture determinations only if required by PARC regulations. Extensive studies on moisture content vs. stability did not show a useful correlation between the moisture content and how stable the vaccine was over its shelf life. Lots that have an excessively high moisture content will undoubtedly be known because of the difficulties experienced during drying and careful monitoring of the titer over its shelf life should be more meaningful. Rejecting product solely on moisture content may mean rejection of good product.

IV. ASSESSMENT AND RECOMMENDATIONS FOR IMPROVING PACKAGING OF VACCINES

The situation in Mali does not really parallel that of the United States in regard to having to have a very attractive container with an attractive label that endusers

identify with. Also mandatory packaging of diluent with a lyophilized product is not required in Mali. The cost of packaging is significant. In the United States, cost of the container exceeds the cost of producing the lyophilized content in the container in many cases but this is justified by the consumer appeal and consumer recognition of ease of administration (as in prefilled syringes). Looked at another way, in Mali the need for CVL to be involved in plain distilled water diluent production may not necessarily be a good idea. Diluent is not a revenue generating item and vaccine costs in Mali barely cover lab production costs at present. It is also a fact that very few vaccine users take time to read a label or note a container design. Costs are therefore of prime consideration. Something that probably the research area must do is investigate the use of killed bacterial products as diluent for the dried products that are distributed in relatively large dosage sizes (i.e. 25 or 50 doses). In order to do this, several things must be considered. In the U.S., the bottle size of the lyophilized portion is usually essentially the same as that of the diluent bottle. A needle with two pointed ends is commonly provided. This needle is first inserted into the diluent bottle and then the other pointed end is thrust through the bottle with dried material. These bottles have good vacuum and the diluent is rapidly pulled into the "dried" bottle. This is certainly more convenient but the smaller Malian "dried" bottle could be reconstituted with a portion of killed product and emptied into the larger "killed" bottle (shaken well and repeated with another portion of the "killed" diluent) so that multiple antigens could be administered with a single inoculation. Another consideration when using this method is to insure the diluent is not viricidal. This is done in quality control procedures by comparing titers of lyophilized product reconstituted with diluent with that reconstituted with sterile distilled water alone after each has been held for two hours at room temperature. Formalin is commonly neutralized by adding sufficient sodium bisulfite to the product to counteract the formalin. See QUALITY CONTROL discussion and also Appendix G for a copy of U.S. regulations for viricidal testing.

The question of smaller dosage sizes can be looked at several ways. The bottle containing the dried products contains only one or two milliliters of fill. Reducing the amount of fill does not appear practical. The easiest way is just to reconstitute the dried portion with less diluent. If you reduced the 50 dose diluent to 10 dose equivalent, in effect, all you have done is raised the amount of virus in the dose five times or raised the titer 0.7 of a log; reducing the 25 dose diluent to 10 doses, you have raised the titer 0.4 of a log. This would appear to be a better approach than reconstituting with a large amount of diluent and discarding the unused doses.

RECOMMENDATION: Producing "combination" products in Mali does not seem to have the potential to be widely accepted either by the lab or by endusers at this time. There seems to be incomplete knowledge as to the feasibility of combining rinderpest vaccination with, for instance, some of the clostridials or with pasteurella products because protection against multiple diseases at the same time does not seem necessary. It is suggested that people in the Diagnostic facilities gain more insight into possible needs for multiple immunizations in the same animal and pursue the possibilities of "combination" products based on this knowledge. This would be a good project for someone in the "marketing" position.

Use of plastic bottles to distribute killed products is an excellent idea. Because of the need to subject dried products to a wide range of shelf temperatures and to stopper the containers under vacuum at the end of drying, the use of plastic bottles for dried products is very rare, if done at all, in the United States.

A visit was made to the Societe Mamadou Sada Diallo, a local manufacturer of plastic products. A discussion was held with the Technical Services Director about the feasibility of manufacture of 500 ml. and smaller size bottles. Two options appear available: 1) CVL buys the mold and SMSD makes bottles based on cost of labor and materials; 2) SMSD buys the mold themselves based on a binding contract sufficiently large to recover the cost of the mold (5 million CFA or about \$15,000; U.S. manufacturers also buy their molds in Europe for about the same price); plus labor and materials. Their machines are the following makes: Bekum (Germany); Mecavil (Serta) (France); and Moretti (Italy). They gave us half a dozen bottles. I kept one and Mr. Boubou will try several others to see if they retain integrity at autoclaving temperatures of 121 C x 30 minutes. SMSD presently uses polystyrene but does not use polypropylene.

RECOMMENDATION: Formulations of the plastic used for 20 ml. and 100 ml. bacterin bottles in the United States and drawings with dimensions are enclosed as Appendix L. This information may be of help in further negotiations with the Bamako plastic bottle manufacturer.

The lab might want to try a small test of holding some product in the plastic bottles presently made by SMSD and after holding for perhaps 6 months, testing the product for potency or for evidences of adverse effects.

The presently used Markem machine for labeling small bottles is adequate in my opinion. The use of locally printed labels for larger bottles also seems adequate. With

the ready availability of labor, the use of automatic equipment for labeling has low priority, in my opinion. If Malians are like most vaccine users, they might read the label once, if at all.

V. ANALYSIS AND RECOMMENDATIONS FOR ADDITIONAL TRAINING

A. OPERATIONS

The idea that someone explore the techniques of true marketing as practiced in more developed countries and adapt them as can be done to situations in Mali is an intriguing one. It might be extremely useful to have one of the CVL staff become more familiar with how the marketing staff of a large U.S. veterinary biologics manufacturer evaluates possibilities for new products, meets with advertising agencies, keeps track of market shares, sets production goals, conducts sales training meetings, and interacts with the research and development and production departments.

Three possible locations that come to mind for training of this sort would be at locations with which I am familiar.

1) A laboratory, Diamond Scientific Company, that has both a large animal and small animal line of products and has recently affiliated with Bayer International, the multinational pharmaceutical manufacturer. By becoming acquainted with people at Diamond, the possibility that trainees could be accepted elsewhere in the Bayer organization in such areas as parasiticides and anthelmintics opens up. 2) At Rhone-Merieux Laboratories in Athens, GA and in Lyons, France. The advantage here is that both English and French are used extensively and there is a special interest in poultry products. A man very interested in poultry problems in less developed countries is Dr. Daniel Gaudry who is presently in Lyons but is in the United States frequently. The production manager in Athens is Mr. Don Hildebrandt, formerly with the large poultry vaccine manufacturer, Dr. Salsbury's Labs in Charles City, IA. Merieux also produces a superior rabies vaccine. 3) The Animal Health Institute in Alexandria, VA, outside Washington, DC. This organization is, in effect, the lobbying group for veterinary vaccine manufacturers in the U.S. People on the staff of AHI have extensive daily working interactions with the largest manufacturers of veterinary vaccines, pharmaceuticals and feed additives in the U.S. Arrangements to accept short term trainees in production methods might also be worked out through AHI.

The development work that is going on at the Plum Island Lab could be of benefit to CVL. As discussed earlier, Dr. Mebus has developed a rinderpest vaccine (or improved the stabilizer and drying methods so that elimination of the cold chain can be considered). Several new products are being looked at. Plum Island will accept

trainees. It certainly seems to be a resource that should be utilized.

Proposed contacts at these organizations are shown in Appendix K.

I believe that some members of the CVL staff are going or have gone to short training sessions at the lab in Nairobi, Kenya. This seems to me a good way to exchange ideas on problems that are uniquely African. If it is possible, I think building and maintaining links with people who are tackling similar problems in Kenya is a good one.

B. MAINTENANCE

Three areas where training may be useful in maintenance are:

1. Virtis Manufacturing Co. for the freeze-dryers.
2. Amsco Sterilizer Co. for sterilizers, steam generators, and for water stills.
3. Clayton Manufacturing Co. for the oil fired steam generators.

In addition, there is a man that has had many years experience in working with freeze-dryers in a veterinary biologics plant. This man's name is Ray Taggart and I understand that he has accepted short-term assignments out of the United States.

C. INSTALLATION OF EQUIPMENT

The installation of equipment is, if possible, best left to field engineers of the manufacturer who can then provide on site training to lab personnel. Unfortunately this service is expensive. In discussions with Virtis, I was told that a field engineer would help with installation but the usual charges are \$75.00 per hour plus expenses from the time of departure to the time of return to the U.S. Because of the long association of CVL people with Virtis machines, coupling installation training with maintenance training for them in the U.S. may be the best course.

Changing the present steam supply system to one with electric steam generators near the sterilizers or replacing the present oil-fired generators may present some engineering and construction problems although Clayton Manufacturing Co. is providing specifications for a complete skid mounted unit that would require only hookups of steam, fuel, water and electricity. Clayton will provide engineers for field installation at \$650 per day plus expenses.

Because the cost of having field engineers on site is quite expensive, it may be more economical to have Malian trainees visit manufacturers in the U.S. Beside the straight installation of sterilizers and steam generators, it would be very valuable for the CVL people to better understand the subtleties of steam generation and pure water preparation.

Complete addresses and phone numbers for institutions mentioned in this section are listed in Appendix K.

VI. MISCELLANEOUS ITEMS

Peace Corps Poultry Project

A man from Iowa, Mr. Rex Adams, has visited Mali twice to help the Peace Corps volunteers and Malians with problems in raising birds. I was impressed with the impact his 40 years of practical poultry raising expertise have had on Malians. It appears that the CVL Poultry Section will be operating an incubator to produce fertile eggs for the newcastle and fowlpox vaccine project. These incubators should be separate from the incubators used for virus production and if all goes as planned should provide chicks that are selectively specific pathogen free. It is unlikely that the incubator will be operating near capacity. Bringing the lab and the Peace Corps projects together to supply healthy chicks could work to the advantage of both. If the CVL poultry people could start good quality chicks and be sure they have been properly vaccinated it would be very valuable to the Peace Corps volunteers. Conversely, by working closely with the local projects, the lab people, especially the Diagnostic Lab will be in a position to get feedback and specimens related directly to problems being encountered in the local area. Fully developed, it could make both endeavors much more meaningful.

RECOMMENDATION: When the incubators become functional, perhaps Dr. Moussa Coulibaly who has worked with the Peace Corps before or Mr. Diawara could contact Mr. Umar Cisse, Agricultural Officer with Peace Corps in Mali to see if a cooperative arrangement can be worked out. When contacted, Mr. Adams was supportive of this idea and was willing to offer advice on sources of cockerels in the U.S. for use in cross-breeding, and offer helps in other areas. He has had experience in 17 countries and should be an invaluable source of good information.

Mineral Blocks for Livestock

There is mounting evidence that mineral deficiencies are causing significant problems in Malian ruminants. Availability of mineral blocks formulated to meet Malian needs would be very useful.

RECOMMENDATION: The following information was obtained from Dr. Eugene Kuhajek of the Morton Salt Co. Research facility in Woodstock, IL (815-338-1800). Most mineral blocks are formed using large presses that use pressures of 20,000 lb/in³. This does not seem practical for Mali. Dr. Kuhajek thought so-called "candy blocks" with a molasses base might be more practical. A press that is capable of forming these blocks is made by Landers Machine Co., 207 E. Broadway, Fort Worth, TX 76104 (817-336-5653). They reportedly cost about \$100,000. A company that has a patented process for casting or pouring these blocks is VMS Co., P.O. Box 406, Montgomery, AL 36101 or 1080 Wilbanks St., Montgomery, 36915 (Attention: Mr. W. Brent Camp, 205-834-6510). These are possible leads for pursuing mineral block manufacture in Mali.

VII. CONCLUSION

There are many positive signs at CVL pointing to an expanded potential that the lab can fulfill. The Diagnostic Section and Research Section has the potential to spot and develop new products that Mali needs. The Production Unit is in need of updating and replacing equipment but the staff is trained with the potential to produce all the good product that Mali (and some surrounding countries) can utilize. There is need to give CVL all the support possible in order for the staff to reach its full potential, increasingly using its own resources. They work under difficult circumstances and must be given encouragement to strive for difficult goals.

ACKNOWLEDGMENTS

If some are omitted, it is unintentional. Everyone contacted has been really helpful.

In Chemonics/Washington: Sally Cameron and Jim Davis

In APHIS, Hyattsville: Bob Miller, Gary Colgrove, Miles Bairey

In NVSL, Ames: John Mitzel, Dale Burge, Bob Clark, Jack Pemberton, Dr. Mebus (Plum Island)

Elsewhere in the U.S.: Alpha Diallo, Darryl Peterson, Rich Betterly, Bill Kelsey, Lou Van Daele, Art Dwight, Laura Olszanowski, Ed Hevern, Kim Murdock, Eugene Kuhajek, Jim Edmiston

In AID/MDST/Chemonics/Bamako: Dick Cook, Richard Pronovost, K. Diarra

At CVL: Dr. Seck, Dr. Simbe, Mr. Kanoute, Mr. Boubou, Mr. Coulibaly, Mr. Sidy Diawara, Dr. Kouyate

At Peace Corps: Tom Elam, Omar Cisse, Rex Adams (Kirksman, IA)

Private Sector/Mali: Dr. Coulibaly

At AID/Bamako: Wayne McDonald, Tracy Atwood, Claudia Cantell, David Atwood, Cheick Drame

A Special Thank You to Dr. and Mrs. Stan Dennis for all the nice things they did.

APPENDICES

A. PRODUCTION PROTOCOLS: See Pages A1 to E8 following.

LABORATOIRE CENTRAL VETERINAIRE BAMAKO
 DIVISION PRODUCTION VACCINS
 VACCINS A VIRUS

LE VACCIN CONTRE LA PESTE BOVINE : (Bovipeste)

Milieux de culture et solutions utilisés en culture cellulaire.

I.- MILIEUX DE CULTURE CELLULAIRE :

1°) HSLS (EARLE HIGH SUCAR, LOW LAH)

| | |
|--|--------|
| NaCl | 28,00g |
| KC1 | 1,60g |
| MgSO ₄ | 0,80g |
| NaH ₂ PO ₄ | 0,52g |
| NH ₄ Cl | 0,20g |
| Glucose | 10,00g |
| * Hydrolysat de lactalbumine | 20,00g |
| Extrait de levure | 0,40g |
| Ca Cl ₂ | 0,80g |
| L. Glutamine | 0,40g |
| L. Acide Glutamique | 1,20g |
| L. Methionine | 0,60g |
| Biotine | 0,004g |
| Acide fcliique | 0,004g |
| Rouge de phenol | 0,080g |
| Eau bidistillée | 4000ml |
| Sérum de veau | 400ml |
| NaHCO ₃ (Bicarbonate de Na) | 1,00g |
| Antibiotique=Pen/Strep | |
| Antifongique= Fungizone | |

2°) EARLES 10X

| | |
|--|--------|
| NaCl | 68,00g |
| Glucose | 10,00g |
| KC1 | 4,00g |
| MgSO ₄ .7H ₂ O | 2,05g |
| NaH ₂ PO ₄ .H ₂ O | 1,44g |
| * CaCl ₂ .2H ₂ O | 2,65g |
| Rouge de phénol | 0,20g |

- * Dissoudre à part et ajouter en dernière position
- Filtrer sur millipore et constituer des aliquotes de 80 ml.
- Congeler.

3°) EARLES MOBILE 5% SERUM BOVIN

| | |
|--------------------|-------|
| Earles 10X | 80ml |
| Sérum bovin | 50ml |
| LAH | 5g |
| NaHCO ₃ | 0,75g |

| | |
|---------------|-------|
| Pen/Strep | 1ml |
| Fungizone | 1ml |
| Eau distillée | 868ml |

Ce milieu à 2 ou à 5% est un milieu d'entretien.

Pour un milieu de croissance ou d'initiation il est recommandé d'utiliser 10% de sérum.

D'autres milieux sont utilisés. A titre indicatif on citera:

Milieu de croissance formule L.C.V.:

MEM Eagle's deshydraté
Sérum de veau 10%

Milieu d'entretien formule L.C.V.

Utiliser le précédent mais avec un taux de 2 à 5% de sérum de veau.

II. SOLUTIONS TAMPONS:

1°) PBS DULBECHO (Phosphate buffered saline)

| | |
|---------------------------------------|--------|
| NaCl | 8,00g |
| KCl | 0,20g |
| * CaCl ₂ | 0,10g |
| MgCl ₂ , 6H ₂ O | 0,10g |
| Na ₂ HPO ₄ | 1,15g |
| KH ₂ PO ₄ | 0,20g |
| Eau distillée | 1000ml |
| pH 7,2 | |

* Dissoudre le CaCl₂ à part et l'ajouter.

2°) PBS sans ions Mg & Ca

| | |
|----------------------------------|--------|
| NaCl | 8,00g |
| KCl | 0,20g |
| Na ₂ HPO ₄ | 1,15g |
| KH ₂ PO ₄ | 0,20g |
| Rouge de phénol | 0,10g |
| Eau distillée | 1000ml |
| pH 7,2 | |

-- 3 --

3°) TAMPON DE HANKS BSS (Balanced Salt Solution)A. Solution à 10X

| | |
|--|--------|
| NaCl | 80g |
| Glucose | 10g |
| KCl | 4g |
| MgSO ₄ , 7H ₂ O | 1g |
| MgCl ₂ , 6H ₂ O | 1g |
| KH ₂ PO ₄ | 0,6g |
| Na ₂ HPo ₄ | 0,42g |
| Rouge de phénol | 0,20g |
| CaCl ₂ , 2H ₂ Ox | 1,85g |
| Eau distillée | 1000ml |

Le CaCl₂, 2H₂O⁺ sera dissout dans une petite quantité d'eau distillée et ajouté en dernière position pour éviter la formation d'un précipité. Filtrer, constituer des aliquotes de 100ml et congeler.

B. NaHCO₃ de Hanks

Dissoudre 14 g de NaHCO₃ dans un litre d'eau et autoclaver 120°C pendant 15 min. Garder à +4°C avant utilisation.

HANKS IX (Solution de travail)

| | |
|--------------------------|--------|
| HANKS 10 X | 100 ml |
| NaHCO ₃ Hanks | 30 ml |
| Eau distillée | 100 ml |

1 L. HALS

(Hanks Ix + antibiotiques + lactalbumine + serum).

| | |
|----------------------------|--------|
| Hanks I X | 800 ml |
| Hydrolysat de lactalbumine | 100 ml |
| Serum de veau | 100 ml |
| Antibiotiques | |

Le pH sera toujours situé entre 7,2 et 7,4. Si nécessaire l'ajuster avec une solution de bicarbonate de sodium 4,4 %

TRYPSINE (1:250, 0,25 %)

| | |
|-----------------|---------|
| Hanks ou PBS Ix | 1000 ml |
| Trypsine 1:250 | 2,5 g |
| pH 7,8 - 8,0 | |

Après 30 minutes à la température du labo filtrer sur millipore et faire des aliquotes de 100 ml. Congeler.

-- 4 --

SOLUTION D'ATV (Solution A de trypsine - versene)

Cette solution est une combinaison de trypsine-versène d'une part et de tampon sans Ca - Mg d'autre part. On pourra donc utiliser le PBS sans Ca et sans Mg pour sa préparation. Cependant la formule du L.C.V. est la suivante :

ATV 10X

Solution A

| | |
|--------------------|-------|
| NaCl | 80g |
| KCl | 4g |
| NaHCO ₃ | 6g |
| Eau distillée | 500ml |

- Dissoudre par agitation fréquente en s'aidant du bain marie à 60°C des sels ainsi énumérés
- Ajouter à la solution saline
Versene (C₁₀H₁₆O₈N₂) 2g
- Homogeneiser en maintenant la solution à 60°C jusqu'à dissolution complète du versène
- Ajouter
Glucose 10g

Solution B

| | | |
|----------------------------------|-------|-------|
| Trypsine | 1:250 | 5g |
| Eau distillée | | 100ml |
| Obtenir solution homogène à 37°C | | |

- Melanger A et B
- Ajouter
Rouge de phenol 10mg
Antibiotiques et antifongiques

Completer le volume à 1000 ml

Filtrer sur millipore et faire des aliquotes de 50 ml.

Solution de travail

| | |
|---------------|--------|
| ATV 10X | 100 ml |
| Eau distillée | 900 ml |
| pH 7,2 - 7,4 | |

Ajuster le pH avec la solution a 4,4 % de NaHCO₃

La solution ainsi obtenue est composée de 0,05 % de trypsine et de 0,025 % de Versene.

HYDROLYSAT DE LACTALBUMINE (HLA)

Solution à 5 % de HLA dans le PBS ou le Hanks à 1X. Chauffer à 80°C pour la dissolution complete du lactalbumine. Repartir à raison de 100 ml/flacon à vis. Autoclaver à la pression de 15 Lbs pendant 15 min. Laisser refroidir et congeler.

L'orqu'un flacon présente des précipités après décongélation, il peut être chauffé pendant quelques minutes.

-= 5 =-

INDICATEURS ET ANTIBIOTIQUES :

Dans la plupart des milieux de culture cellulaire du LCV on inclut non seulement des antibiotiques et des antifongiques pour prévenir les contaminations mais aussi un indicateur coloré pour ajuster le pH et renseigner sur le métabolisme des cellules en culture par le virage de l'indicateur. C'est ainsi qu'on citera à titre indicatif les concentrations recommandées pour :

- Les antibiotiques

| | | |
|-----------------|-----------|-----------------|
| Penicilline | 100 - 200 | UI/ml de milieu |
| Streptomycine | 50 - 100 | /ml |
| Chloramphénicol | 10 - 20 | /ml |
| Aureomycine | 10 | |
| Tétracycline | 10 | /ml |
| Bacitracine | 10 | /ml |

- Antifongique

| | | |
|-------------|----------|----|
| Mycostatine | 50 - 100 | UI |
| Fungizone | " | |

- Indicateur

| | |
|-----------------|----------|
| Rouge de phenol | 0,002g/l |
|-----------------|----------|

- STABILISATEUR :

Formule PLOWRIGHT

Solution A :

| | |
|---------------|-----------------------------------|
| Sucrose | 400g |
| LAH | 200g (hydrolysat de lactalbumine) |
| Eau distillée | 4000ml |

Dissoudre à 56°C et filtrer sur millipore

Faire des aliquotes de 1000 ml et conserver à +4°C.

Solution B :

| | |
|---------------|--------|
| Gelatine | 200g |
| Eau distillée | 1000ml |

Dissoudre à 56°C. Repartir et stériliser à 15psi pendant 20 min. Conserver à la température du labo ou à 37°C.

Solution finale :

| | |
|------------|---------|
| Solution A | 900 ml |
| Solution B | 100 ml |
| Vaccin | 1000 ml |

Homogénéiser avant répartition du vaccin

éviter la solidification de la gelatine.

Formule L.C.V.

Solution A - Lactose Glutamate

| | 1 litre | 4 litres |
|---|---------|-------------|
| Lactose | 200g | 000g _____ |
| Mono Potassium Phosphate ($K H_2 PO_4$) | 1,98g | 7,92g _____ |

-- 6 --

| | | | |
|--|--------|--------|-------|
| Dipotassium Phosphate (K ₂ H PO ₄) | 4,70g | 18,80g | _____ |
| Monopotassium Clutomate | 1,92g | 7,68g | _____ |
| Eau bidistillée | 1000ml | 4000ml | _____ |

Solution B NZ Amine AS

| | 1 litre | 4 litres | |
|-----------------|---------|----------|-------|
| NZ - Amine AS | 200g | 800g | _____ |
| Eau bidistillée | 1000g | 4000g | _____ |

Solution finale :

| | |
|------------|---------|
| Solution A | 600 ml |
| Solution B | 600 ml |
| Vaccin | 1200 ml |

Homogeneiser et repartir.

Formule Dakar - Hann

| | |
|---|------------|
| Solution de Hanks avec 10 % de saccharose) | 1 partie |
| Lait écremé en poudre 20 %) | 1 partie |
| Vaccin | - 1 partie |

PREPARATION DU VACCIN :

A. Culture de Cellules d'Explants Primaires :

La méthode, simple et satisfaisante permet d'obtenir des cellules du type épithéial plus réceptives aux virus que celles du type fibroblastique. Les étapes essentielles sont les suivantes.

1. Prélever les reins d'un foetus obtenu immédiatement après abattage de la vache gestante.
2. Récolter le cortex, découper en morceaux avec des ciseaux.
3. Mettre les fragments dans un Earlenmayer de 500 ml et laver 3 fois avec un milieu de culture (PBS, MEM, EARLE ou HANKS).
4. Après lavage mettre 1 vol. de fragments de rein en suspension dans 4 volumes de trypsine à 0,25 %.
5. Placer un barreau magnétique dans le ballon
6. Déposer le ballon sur un agitateur électromagnétique et permettre une agitation très faible de la suspension rénale.
7. Laisser la trypsinisation évoluer à la température du labo pendant une heure (1 H).
8. Vider le surnageant et rincer les fragments de tissu 3 fois avec une solution de trypsine à 0,25 %. (Solution de travail)

Le but de cette opération est d'éliminer toutes les substances toxiques pouvant apparaître au cours de la trypsinisation précédente.

-- 7 --

9. On mettra finalement une quantité nécessaire de trypsin à 0,25 % (4 volumes de trypsin pour 1 volume de tissu à digérer) et permettre ainsi la trypsination d'évoluer à la température de la salle pendant une heure comme dans les phases 6 et 7.
10. Filtrer la suspension cellulaire à travers une épaisseur de gaz réalisée à cet effet dans un récipient stérile. Reprendre les opérations 9 et 10 si l'on désire obtenir un très grand volume de cellule.
11. Mettre la suspension cellulaire ainsi filtrée dans des godets de centrifugation.
12. Centrifuger à 600 tours à la minute pendant 5 minutes.
13. Vider le surnageant et remettre les cellules en suspension dans un milieu nutritif. Centrifuger une nouvelle fois à 600 tours à la minute pendant 3 mn, ceci pour éliminer l'excès de trypsin et les débris de cellule.
14. Faire une suspension cellulaire à raison d'un volume du culot cellulaire (environ 10^5 cellules/ml) pour 200 volumes de milieu nutritif à 10 % de sérum bovin.
15. La répartition se fait à raison de 100ml de suspension cellulaire par flacon roux de 150 cm² incubation 35 - 37°C.
16. Le changement de milieu se fera dès que le pH aura baissé.

Ceci sera indiqué par le virement de la couleur rouge au jaune du rouge de phenol. Généralement cela apparaît vers le 3e jour lorsque les cellules présentent des granulations.

17. Après 6-8 jours il y'a un tapis confluent de cellules épithéliales (épithéloïdes) sur toute la paroi interne du flacon en contact avec la surface plane de l'étagère. Les flacons sont ainsi prêts pour l'inoculation du virus ou pour un passage dans d'autres flacons.

Quand bien même d'autres lignées cellulaires peuvent servir de support au virus de la peste bovine (cellules vero, cellules de reins d'ovin, cellules testiculaire etc...) les normes internationales limitent le nombre de passage à 10 tant pour les cellules d'explants primaires que secondaires. Après le 10e passage les cellules ont tendance à subir des transformations néoplasiques.

CULTURES CELLULAIRES DE PREMIERE EXPLANTATION SENSIBLES AU VIRUS BOVIPESTIQUE UTILISEES AU L.C.V. DE BKO :

Dans notre laboratoire les tissus communément utilisés sont d'origine

- bovine : rein foetal, rein de veau nouveau né, testicules bovins
- ovine : rein foetal, testicules.

Cependant les reins de porc, de la chèvre, du chien, du hamster, les cellules embryonnaires du poulet aussi bien que les cellules amniotiques de l'homme peuvent être utilisées.

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Lignée cellulaires sensibles au virus bovípestique et leur appellation :

Rein de bovin : MDBK
 Rein de porc : PK
 Rein de singe : Vero
 Rein de Hamster : BHK
 Cellules néoplasiques humaines : HE LA

Toutes ces lignées cellulaires supportent le développement du virus pestique qui se manifeste par un effet cytopathogène (ECP)

L'effet cytopathogène se caractérise par des cellules arrondies, stellaires qui, plus tard deviennent des cellules géantes amitotiques multinucléées ou syncitia, qui accumulent des substances éosinophiles dans le cytoplasme.

Ces cellules géantes sont généralement le résultat de fusion de cellules adjacentes infectées.

Dans les cultures cellulaires la formation des plaques ou zones claires au sein du tapis cellulaire confluant est une conséquence de la mort des cellules.

B. Production du Vaccin et Divers Tests de Contrôle :

La procédure suivante est effective pour la production du vaccin antipestique atténue, sur culture cellulaire.

Des cellules rénales d'explants primaires sont préparées conformément à la description de la section A. Généralement 12 flacons Roux suffisent pour produire environ 100.000 doses de vaccin.

Le 3^e jour après l'explantation des cellules, le milieu est changé et chaque flacon est infecté avec une semence de virus bovípestique de culture cellulaire atténuee. (Souche Kabété 0 au 90^e passage)

2×10^4 (DICT 50) Dose infectante 50 % de culture tissulaire du virus pestique bovin atténue est utilisée. (2ml du stock de la semence ayant un titre de 10^4 DICT/1ml par flacon). L'inoculum de virus est incorporé au milieu qui remplacera le précédent dans le changement de milieu de culture. Chaque flacon Roux en recevra 100 ml. Il sera indispensable de s'assurer d'abord si les cellules poussent normalement avant l'inoculation. Au cas contraire l'on attendra plus tard. Eviter par ailleurs de manipuler d'autres virus le jour où l'on doit inoculer des cellules avec le virus pestique atténue. Ceci nous mettra à l'abri d'autres contaminations virales.

Le milieu nutritif des cellules ainsi infectées sera changé le 5^e ou le 4^e jour après l'inoculation.

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A ce moment l'effet cytopathogène du virus pestique commençera à apparaître. 3 à 4 jours plus tard l'effet cytopathogène (ECP) devra s'étendre à 80-90 % des cellules. A ce moment la récolte a lieu en collectant le liquide de tous les flacons dans un grand récipient. Un volume égal de stabilisateur sera ajouté à ce liquide pour en faire un vaccin. Le produit obtenu sera conditionné en flacons de type penicilline à raison de 1ml/f1. et lyophilisé immédiatement. Le vaccin sera alors conservé dans un congélateur à -20°C avant livraison.

C. Tentative de Programme pour la Production du Vaccin Antipestique :

Jour 1 : Extraction des cellules rénales

Jour 3 : Observation du tapis cellulaire. Il doit être couvert au 1/3 au moins.

Jour 4 : Lorsque la culture est bonne, changer le milieu de culture et inoculer les cellules avec le virus bovípestique.

Jour 7 : Les cellules doivent montrer un début d'effet cytopathogène. Changement de milieu.

Jour 8 : Lavage et stérilisation des flacons de conditionnement. Préparation du lyophilisateur.

Jour 9 : Le stabilisateur et les flacons de conditionnement déjà prêts seront stockés au réfrigérateur ou dans une chambre froide.

Jour 10 : Lorsque l'effet cytopathogène est presque complet récolter le liquide et lui ajouter une quantité égale de stabilisateur. Répartir et lyophiliser.

D. Préparation et Contrôle de la Semence :

Dans le but d'éviter d'innombrables passages du virus il est recommandé qu'un lot de semences soit préparé chaque 6 mois. Le stock sera gardé de préférence à -70°C. La semence fera l'objet d'un test de sterilité et de spécificité par le test de séro-neutralisation sur culture cellulaires avant l'utilisation et doit avoir un titre d'au moins 5 DICT₅₀

Les semences sont en général sous forme lyophilisées et stockées à -70°C. La semence de travail quant à elle est un produit liquide reconstitué à partir du précédent aussi stockée à -70°C. Cette dernière forme donne un effet cytopathogène (ECP) plus rapide (3 - 4 jours). Dans notre laboratoire le titre de la semence reste toujours autour de 10⁶ DICT₅₀.

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E. Test d'Innocuité sur Bovins Cobayes et Souris :

- Utiliser des animaux neufs de plus d'un an
- Procéder à un screening test pour déterminer la réceptivité des bovins d'expériences. En l'occurrence on prélèvera le serum de ces animaux aux jours J0 avant l'inoculation du vaccin et J21 pour rechercher la présence des anticorps anti-pestiques. Par la même occasion d'autres anticorps viraux pourront être détectés.
- * Reconstituer 1 flacon de VT de 100 doses vaccinales ou 2 flacons de 50 doses.
- * Inoculer 2 bovins par voie sous-cutanée derrière l'épaule avec le produit ainsi reconstitué équivalent à 100 doses le 1er jour après prélèvement du sang.
- * La température rectale matinale sera enregistrée pour chaque animal. D'autres symptômes seront également enregistrés et cela pendant 3 semaines.
- * Le résultat est satisfaisant si et seulement si les animaux ne présentent pas de réactions cliniques anormales et qu'une seroconversion est constatée après les trois semaines.

Le test d'innocuité peut aussi être réalisé sur des cobayes et même sur des souris dans le but d'éviter des dépenses disproportionnées (coût élevé des bovins) et surtout qu'à l'état actuelle des choses il est difficile d'obtenir des animaux n'ayant pas d'anticorps antibovipestiques (vaccinés pour ceux sevrés et immunité colostrale pour ceux à la mamelle).

On utilisera donc :

5 cobayes dont 1 témoin

- * Reconstituer plusieurs flacons de VT (entre 5 et 10) avec 1ml d'eau distillée stérile par flacon.
- Faire un mélange du produit de reconstitution.
- * Injecter 0,5 ml à chaque cobaye par voie intraperitoneale (IP)
- * Au cas où les souris seraient choisies, injecter 0,1 ml IP.
- * Période d'observation 21 jours

Résultat :

Le test est concluant si tous les animaux restent en bon état de santé jusqu'à la fin de la 3ème semaine.

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F. Test d'Efficacité :

- 5 bovins receptifs de plus de 12 mois dont la réceptivité a été déterminée par séronutralisation (SN) parmi lesquels on choisira :
- 2 bovins témoins
- 3 des bovins seront vaccinés avec le vaccin à tester à raison de 1ml par animal par voie sous-cutanée.
- 3 semaines plus tard tous les animaux vaccinés aussi bien que ceux servant de contrôle (témoins) seront inoculés avec un minimum de 10^3 DL₁₀₀ de virus sauvage en sous-cutané.

Résultats :

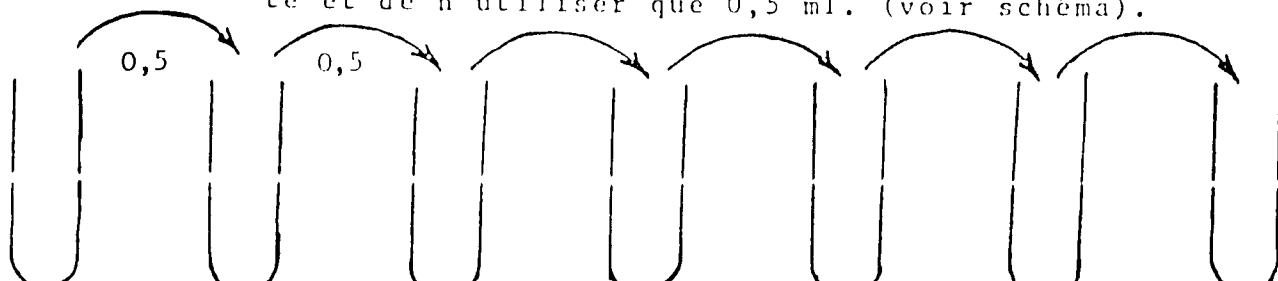
Le test est satisfaisant si les animaux vaccinés restent protégés et les témoins présentent les symptômes de la maladie à 100 %.

G. Titrage du Vaccin en Culture Cellulaire :

Il se fait en triplicata sur cellules rénales d'embryon de bovin. La moyenne des trois résultats sera considérée comme le titre du vaccin. L'écart entre les trois manipulations devra être minime au risque de reprendre le test. La FAO retient un titre minimum de $10^{2.5}$ DICT₅₀/dose vaccinale pendant que notre laboratoire requiert un titre de 10^3 DICT₅₀/dose à la sortie du labo.

TECHNIQUE DE TITRAGE DU VIRUS VACCIN :

- Disposer de 6 tubes marqués 10^{-1} à 10^{-7}
- Mettre 8 ml de PBS dans le 1er marqué 10^{-1}
- Mettre 4,5 ml de PBS dans chacun des tubes restant (10^{-2} - 10^{-7}).
- Prendre plusieurs flacons de VT (10 flacons)
- Reconstituer la pastille de chacun avec 2ml d'eau distillée après avoir testé le vide.
- Mélanger le contenu de ces 10 flacons dans un Erlenmeyer et le mettre sur glace.
- Prelever 2ml de ce mélange à l'aide d'une pipette et le transferer dans le 1er tube pour obtenir une suspension à 10^{-1}
- Faire des dilutions décimale à extinction avec les autres tubes en prenant soin d'homogénéiser le contenu du tube précédent, de changer chaque fois de pipette et de n'utiliser que 0,5 ml. (voir schéma).



| PBS | 8ml | 4,5 | 4,5 | 4,5 | 4,5 | 4,5 | 4,5 |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| Vaccin(Pool) | 2ml | 0 | 0 | 0 | 0 | 0 | 0 |

| Dilution | 10^{-1} | 10^{-2} | 10^{-3} | 10^{-4} | 10^{-5} | 10^{-6} | 10^{-7} |
|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|

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- Inoculer 3 boîtes de cellules normales par dilution en utilisant soit 0,2 ml soit 1ml par boîte.
- Incuber à 37°C pendant 10 jours.
- Examiner les boîtes tous les jours à partir du 3e jour et éliminer celles qui présenteront un effet cytopathogène en prenant soin d'enregistrer les résultats.
- Changer le milieu de culture avant la conclusion du test chaque fois que cela s'avérera nécessaire (milieu trop acide).
- Au 10^e jour calculer le titre du vaccin par la méthode de reed and Muench ou de SPEARMAN - KARBER.

Exemple de Calcul du titre par la méthode statistique des totaux cumulatifs. (Reed et Muench)

| Dilution | ECP + | ECP - | CUMUL + - | PROPORTIONS | POURCENTAGE D'INFECTION |
|------------------|----------|----------|--------------|-------------|----------------------------|
| 10 ⁻¹ | 3 | 0 | 13 0 | 13/13 | 100 |
| 10 ⁻² | 3 | 0 | 10 0 | 10/10 | 100 |
| 10 ⁻³ | 3 | 0 | 7 0 | 7/7 | 100 |
| 10 ⁻⁴ | 2 | 1 | 4 1 | 4/5 | 80 |
| 10 ⁻⁵ | 1 | 2 | 2 3 | 2/5 | 40 |
| 10 ⁻⁶ | 1 | 2 | 1 5 | 1/6 | 16 |
| 10 ⁻⁷ | 0 | 3 | 0 8 | 0/8 | 0 |

- Le facteur de dilution dans notre cas est 10
Le log de 10 = 1
 - Si l'inoculum est de 0,2 ml alors pour ramener les résultats à 1ml le facteur de multiplication sera 5 et le log de 5 est 0,7
 - Calculer la distance proportionnelle :
- $$\frac{(\% \text{ d'infecteds immédiatement supérieurs à } 50\%) - 50\%}{(\% \text{ d'infecteds immédiatement supérieurs à } 50\%) - (\% \text{ d'infecteds imm. infes. à } 50\%)}$$

Ex

$$dp = \frac{80 - 50}{80 - 40} = 0,75$$

- Multiplier la dp par le log du facteur constant de dilution (10) qui est de 1 soit $0,75 \times (-1) = -0,75$
- Ajouter ce résultat au log de la dilution donnant un pourcentage de tubes infectés immédiatement supérieur à 50 %. Ici ceci est 10^{-7} donc on aura :

$$(-4) + (-0,75) = -4,75$$

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- Dose infectante 50 % : $10^{-4,75}$
- Titre viral : $10^{-4,75}$ DICI₅₀/0,2 ml
- Pour ramener le titre à 1ml on ajoutera au titre viral le log du facteur de multiplication (log de 5 = 0,7) soit donc un titre de $10^{5,45}$ DICI₅₀/50 doses
- Pour ramener le résultat à 1 dose vaccinale soit 1 ml soustraire le log du facteur de division de 50 qui est 1,7.
- Finalement le titre de notre VT est de :

$$5,45 - 1,7 = 3,75$$

Titre viral : $10^{3,75}$ DICI₅₀/ml

CONCLUSION :

Ce lot de vaccin en ce qui concerne le titre est dans les normes.

Une autre méthode de calcul du titre qui n'utilise pas les totaux cumulatifs est pratiquée c'est celle de :

SPEARMAN KARBER

La formule suivante est appliquée :

$$- \frac{1}{-} \left(\frac{\text{Somme des pourcentages de mortalités}}{100} \right) - 0,5$$

$$\text{Ex : } - \frac{1}{-} \left(\frac{100 + 100 + 100 + 80 + 40 + 16 + 0}{100} \right) - 0,5$$

$$= - \frac{1}{-} \left(\frac{436}{100} \right) - 0,5$$

$$- \frac{1}{-} 4,36 + 0,5 = 4,86$$

Le titre viral pour 50 doses : $10^{4,86}/0,2\text{ml}$

Pour ramener le titre à 1ml d'inoculum : + 0,7

Pour ramener le titre à une dose vaccinale - 1,7

$$\text{Titre viral final} = 10^{3,86}\text{DICI}_{50}/\text{ml}$$

Les résultats des deux méthodes ne s'écartent pas beaucoup l'un de l'autre et toutes deux jouissent d'une certaine précision.

LE TEST DE SERONEUTRALISATION :

Ce test permet de quantifier la propriété de neutralisation de l'infectivité par les anticorps (Ac) résultant de la tripleinteraction Antigène (Ag) Anticorps - Cellules de l'hôte. Dans notre cas spécifique c'est une mesure de la concentration de l'Ac.

L'une des deux variantes peut être utilisée à savoir :

1°) concentration constante du virus
concentration décroissante de serum

2°) concentration variable du virus
concentration constante de serum

Seul le 1er cas sera traité.

MATERIEL :

Virus bovípestique, serums avant vaccination (Jo) et post vaccination au jour J21, Cellules rénales en tube roller ou en boîtes de 75 cm² des tubes de dilution pipettes, diluant.

PROCEDURE :

- Faire des dilutions décimales de 10^{-1} à 10^{-10} du virus bovípestique
- Parallèlement à ces dilutions de virus faire une rangée de tubes vides dans lesquels on mettra 0,3 ml de diluant.
- Ajouter à ce diluant un volume égal de suspension virale qui lui correspond.

Mélanger très bien le contenu de chaque tube avec une pipette au moins 10 fois.

Utiliser une nouvelle pipette pour chaque dilution

PREPARATION DU MELANGE SERUM VIRUS :

- 1.- Une troisième série de tubes parallèlement placées aux autres recevront 0,3 ml chacun de serum normal avant vaccination (Jo)
- Ajouter à chacun des tubes de serum un volume égal de virus dilué qui lui correspond
- Mélanger le contenu au moins 10 fois

- 2.- Reprendre le même processus qu'au paragraphe 1 avec le serum convalescent ou serum au jour J21.

- 3.- Incuber à la température du labo pendant 30 minutes

INOCULATION DES CELLULES :

1. Inoculer 5 boîtes de cellules (boîtes de roux de 75 cm²) à raison de 0,1 ml par boîte.
On peut utiliser la même pipette à condition de commencer par la dilution la plus forte (10^{-10}).
2. Reprendre le même processus avec les dilutions du virus

INTERPRETATION DES RESULTATS :

1. Observer les flacons tous les jours pendant une semaine
Enregistrer l'effet cytopathogène
2. Calculer l'indice de neutralisation.

| | <u>TUBES</u> | | | | | | | | | |
|------------------------------|--------------|-----------|-------|-----------|-------|-----------|-------|-----------|-------|-----------|
| | : 1 : | : 2 : | : 3 : | : 4 : | : 5 : | : 6 : | : 7 : | : 8 : | : 9 : | : 10 |
| <u>DILUTIONS DU VIRUS</u> | | | | | | | | | | |
| Diluant (ml) | : | 4,5 | : | 4,5 | : | 4,5 | : | 4,5 | : | 4,5 |
| Virus (ml) | : | 0,5 | : | 0 | : | 0 | : | 0 | : | 0 |
| Dilutions (ml) | : | — | — | — | — | — | — | — | — | — |
| Dilution du virus | : | 10^{-1} | : | 10^{-2} | : | 10^{-3} | : | 10^{-4} | : | 10^{-5} |
| <u>CONTROLES VIRUS</u> | | | | | | | | | | |
| Dilution virus (ml) | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 |
| Diluant (ml) | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 |
| Serum | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 |
| <u>MELANGES SERUMS VIRUS</u> | | | | | | | | | | |
| Dilution virus (ml) | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 |
| Serum | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 | : | 0,3 |

* Les quantités de serum et de virus peuvent variées selon les besoins, cependant les ingrédients seront mélangés en parties égales.

Le test de neutralisation (IN) consiste à faire des titrages parallèles du virus en présence et en l'absence de serum inconnu ou serum à tester.

Déterminer pour chaque groupe le point final 50 pour cent. La différence de titre en log entre le virus témoin d'un côté et le titre du mélange virus serum de l'autre côté représente l'index de neutralisation du serum (IN).

Exemple :

Le titre du virus contrôle = $10^{4,8}$

L'échantillon suspect = $10^{2,3}$

Différence (IN) = $10^{2,5}$

L'index de neutralisation est antilogue de $10^{2,5} = 3000$.

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VACCIN ANTI-PERIPNEUMONIQUE
 -:-:-:-:-:-:-:-:-:-:-:-:-

Nom de code: Péri-T₁

TECHNOLOGIE DE LA PRODUCTION

A. PREPARATION DU MILIEU LIQUIDE

Le Laboratoire Central Vétérinaire utilise le milieu tryptose aussi appelé milieu de Gourlay.

| | |
|----------------------------------|---------|
| Tryptose | 20 g |
| Glucose ou Dextrose | 5 g |
| NaCl | 5 g |
| Na ₂ HPO ₄ | 2,5 g |
| Extrait de levure | 1 g |
| Eau distillée | 1000ml. |

Dissoudre les ingrédients en s'aidant d'un chauffage doux (vapeur ou bain marie) ou d'un agitateur magnétique.

Ramener la température du milieu à celle de la salle. Pendant ce temps inactiver le sérum de cheval (56°C pendant 30 minutes). Le volume de ce serum sera le dixième ou le 15ème du volume total du milieu Gourlay. Dans notre cas nous ajouterons:

| | |
|--------------------------|----------|
| Glycerine | 5ml |
| Sérum de cheval inactivé | 150ml |
| Pénicilline | 100UI/ml |

Ajuster le pH à 7,6 - 8,0.

Filtrer et repartir dans des récipients désirés selon les besoins.

Incuber à 37°C pendant 24-48h pour en vérifier la stérilité.

- Pour la production du vaccin T₁ le volume du liquide après répartition devra être inférieur au volume de l'air dans le ballon de production afin d'assurer une bonne aérobiose.
- Pour le repiquage de la souche, le milieu filtré sera reparti à raison 100, 200, 250 ou 300ml/flacon.
- Pour le dénombrement des particules viables en bouillon ou pour l'isolement des mycoplasmes, la répartition se fera à raison de 9ml par tube.
- La conservation du milieu se fera à +4°C.

B. TECHNIQUE DE PREPARATION

1. L'inoculum

Il doit être prêt trois jours avant le jour de l'inoculation et sera gardé à l'étuve. La semence préparée fera l'objet d'un test de stérilité sur gelose au sang, bouillon thioglycollate et tryptose soy broth (TSB)

2. Le milieu de production

Il sera lui aussi éprouvé à 37°C pendant 24-48h à l'avance.

3. Programme de production

Il se résume comme suit :

Jour 1. Repiquage de la souche T₁ M44 à partir de la "banque congelée"

Le repiquage consiste à faire des dilutions décimales de la pastille de la souche lyophilisée reconstituée dans 1ml d'eau distillée stérile.

Les dilutions seront incubées à 37°C pendant 72 heures.

Jour 4. Contrôler la pureté et l'identité de la souche par contraste de phase et la coloration gram pour la première et la technique d'immunofluorescence pour la seconde.

Reperer la plus forte dilution indiquant une croissance et repiquer la culture sur bouillon de tryptose à raison de 1ml de culture pour 100ml de milieu.

-Incuber à 37°C pendant 48 heures.

Jour 6. Contrôle de pureté des aliquotes de 100ml (gram). Ensemencer les milieux de production. Dans ce cas précis on utilisera 200ml de culture pour 4000ml de bouillon. Incubation statique à 37°C pendant 24 heures.

Jour 8. Mettre les ballons de production sous agitation magnétique modérée. Cette technique permet d'activer la croissance des mycoplasmes.

Jour 8. Récolte du vaccin et addition d'un stabilisateur à raison de par litre de culture, réfrigerer les ballons de culture par immersion en glace fondante. Bien agiter pour homogénéiser.

* Le stabilisateur est le lait écrémé sec commercial.

NB: Il est recommandé avant addition du stabilisateur de faire un contrôle de pureté rapide (gram, contraste de phase

- Répartition du vaccin ainsi obtenu dans des flacons de type pénicilline à raison de 2ml par flacon.
- Lyophilisation immédiate ou réfrigération rapide ne dépassant pas 24h si l'on n'est pas prêt.
- Le stockage du produit prêt à l'utilisation sera fait à 20°C.
- La chaîne de froid ne devra pas être interrompue dès la récolte du vaccin jusqu'au moment de son utilisation sur le terrain si l'on veut obtenir des résultats satisfaisants. Cependant, le fait de garder des flacons bouchés sous vide non capsulés au congélateur en attendant de les capsuler peut conduire à des résultats facheux si l'on sait que les bouchons se retractent, par conséquent il se produit un microfuitage.

C. CONTROLE DU VACCIN

Chaque lot de vaccin sorti est soumis aux tests suivants:

1. Contrôle du vide

Il se fera à l'aide du vérificateur à haute fréquence sinon à l'aide d'une seringue ou simplement par ouverture d'un flacon. A l'ouverture la pastille sautille.

2. Contrôle de pureté ou absence de contaminants bactérien et fongique. Ensemencement :

- en milieu aérobie liquide (TSB)
- en milieu anaérobie liquide, le bouillon thioglycollate est indiqué.
- sur gelose au sang
- sur sabouraud

Tous les milieux ainsi inoculés doivent rester stériles après 10 jours d'incubation.

Les mêmes milieux seront ensemencés pour tester le solvant (eau distillée stérile).

3. Contrôle d'identité ou présence de M. mycoides

Sur le produit fini il faudrait isoler à nouveau le mycoplasme (milieu de numération ou gelose). L'identification se fera par la méthode d'inhibition de croissance. Dépot de disques imprégnés d'antiserum mycoïdes sur gelose après ensemencement de celle-ci.

4. Contrôle d'innocuité

Reconstituer le vaccin de manière à obtenir une dose vaccinale par inoculum et inoculer par voie intrapéritoneale:

- . 2 souris témoins avec le solvant à raison de 0,25ml
- . 2 souris avec du vaccin reconstitué
- . 2 cobayes mâles avec du vaccin reconstitué.

Conclusion du test

Si en 7 jours d'observation 90 pour 100 des animaux inoculés restent vivants alors le test est concluant.

5. Titrage

- . Il se fera à partir du vaccin reconstitué à la dose vaccinale avec le solvant approprié.
- . Faire des dilutions décimales de -1 à 12 en bouillon mycoplasme
- . Ensemencer 1ml de chacune des dilutions de 10^{-6} à 10^{-12} dans cinq tubes contenant 9ml de bouillon mycoplasme.
- . Incuber pendant 10 jours à 37°C tout en appréciant la croissance dès le 4^e jour.
- . Noter le nombre de tubes ayant poussé
- . Déterminer le titre par la méthode de calcul du "Most probable number (MPN) par la table de Mac CRADY
- . Aussi peut-on utiliser la méthode de Reed et Munch.

Résultats

Dans notre laboratoire un titre minimum de 10^7 unités viables par dose vaccinale est exigé.

PROGRAMMATION DE LA PRODUCTION DU VACCIN
ANTI- CHARBON BACTERIDIEN
NOM DE CODE : RNTHRAVAC

PROTOCOLE

- Jo Préparation et stérilisation du milieu de culture (gelose ordinaire) dans les boîtes de Roux
- J2 Repiquage de la souche mère Bacillus anthracis sur gelose dans les boites de petri.
- J3 Ensemencement de l'inoculum
- J4 Ensemencement des boîtes de Roux après le contrôle de pureté de l'inoculum.
- J5 Préparation et stérilisation du matériel pour la recolte
- J6 Recolte des boîtes de Roux après contrôle de pureté
- J8 Agitation de la souche-mère glycerinée à la température ambiante
- J9 Titrage de la souche-mère
Destruction des formes végétatives —> agitation à +4°C
- J10 Préparation du matériel pour la repartition du vaccin :
stérilisation des flacons; glycerine, eau physiologique, seringue de repartition.
- J11 Repartition du vaccin
test de stérilité et de pureté
contrôle du titre.

VACCIN ANTI-CHARBON BACTERIDIEN
 NOM DE
 CODE : ANTHRRAVAC

I. SOUCHE

Bacillus anthracis 34F2 Weybridge, souche acapsulogène avirulente.

II. VACCIN :

Suspension saline glycerinée à 50 p 100 de spores de B. anthracis titrant 2.10^7 spores/ml (dose vaccinale 1 ml). On admet que la dose vaccinale doit se situer entre 10^7 et 2.10^7 spores vivantes.

III. FABRICATION DU LOT DE SEMENCE :

On évitera d'effectuer plus de 3 repiquages à partir du tube de souche-mère.

| | |
|----------------------------|---|
| Tube de souche lyophilisée | |
| 1ère génération..... | Boîtes de Roux |
| 2 ème génération..... | Flacons de réserves lyophilisée |
| 3ème génération..... | ensemencement de bouillon ordinaire = lot de semence servant d'inoculum |

Le flacon de réserve est repris dans 2 à 3 ml de bouillon qui serviront à ensemencer autant de tubes de bouillon qu'il faut par la suite.

IV. CONTROLE DES CARACTERES DE L'INOCULUM APRES 24 HEURES A 37°C

Caractère du bouillon, état frais, gram

V. PREPARATION DU VACCIN :

1. Préparation du milieu

| | |
|---------------------------------------|---------|
| Bactotryptose..... | 10grs |
| Extrait de levure..... | 2grs |
| KH ₂ PO ₄ | 1gr |
| K ₂ HPO ₄ | 5grs |
| Bactoagar..... | 30grs |
| Eau distillée..... | 1 litre |

1.1. Dissoudre les sels dans l'eau distillée tiède puis le bactotryptose et la levure.

1.2. Filtrer sur papier

- 1.3. Porter au bain marie bouillant et ajouter petit à petit le bactoagar.
- 1.4. Ajuster le pH à 7,3 avec de la soude
- 1.5. Repartir environ 120 ml par boite de Roux
- 1.6. Autoclaver à 120°C pendant 40 minutes
- 1.7. Mettre les boites de Roux à plat à leur sortie de l'autoclave (gelose en bas)
- 1.8. Placer le lendemain, les boites de Roux à 37°C (gelose en haut) ; elles y resteront pendant 3 à 4 jours.

2. Ensemencement des boites de Roux

- 2.1. Eliminer l'eau de condensation en excès dans les boites de Roux.
- 2.2. Ensemencer chaque boite de Roux avec 5 à 10 ml d'inoculum
- 2.3. Etaler la culture sur toute la surface de la gelose
- 2.4. Laisser les boites de Roux gelose en bas pendant 1 heure
- 2.5. Placer les boites de Roux à 37°C après les avoir retournées
- 2.6. Au bout de 48-72 heures la sporulation est considérée comme terminée
- 2.7. Ecarter les lots n'ayant pas sporulés.

3. Récolte des boites de Roux

Il faut auparavant contrôler le degré de sporulation et la pureté (gram, état frais, bouillon et gelose ordinaires)

- 3.1. Introduire 50 à 60 ml d'eau physiologique stérile dans chaque boite de Roux
- 3.2. Laisser le temps de décoller si le décollement est difficile, ajouter 1 à 2 cm³ de billes de verre.
- 3.3. Filtrer sur un entonnoir muni de gaz stérile
- 3.4. Toutes les billes doivent sortir des boites. Rincer le gaze avec de l'eau physiologique stérile.
- 3.5. Ajouter à la récolte un volume égal de glycérine stérile
- 3.6. Agiter fortement la suspension mère glycérinée ainsi obtenue
- 3.7. La suspension mère est placée à la température ambiante pendant 48-72 heures environ pour faire 60° pendant 20 min pour détruire les formes végétatives si l'on veut continuer la suite des manipulations aussitôt.
- 3.8. Contrôle de la pureté de la suspension mère : état frais, gram, bouillon et gelose ordinaire.

4. Titrage de la suspension mère glycérinée

- 4.1. Agiter la suspension mère toute la nuit précédente le titrage en chambre froide (+4°C)
- 4.2. Repartir le diluant (serum physiologique glyceriné au demi) dans 4 erlenmeyer de 250 ml - 90 ml par contenant
1 erlenmeyer de 250 ml - 80 ml
3 tubes de 16 - 9 ml par tube
1 tube de 16 contenant la suspension mère-glycerinée
- 4.3. Effectuer les dilutions 10^{-2} , 10^{-4} , 10^{-6} , $2 \cdot 10^{-7}$ et 10^{-7} (cf schéma).

- 4.4. Ensemencer 5 boites de Petri par dilution

- 4.5. Lecture des boites après 24h, 48 h, 72 h et 4 jours d'incubation à 37°C

On déterminera :

- le nombre moyen de bactéries

- l'erreur absolue et l'erreur standard relative

5. Ajustement du titre de la suspension mère à $2 \cdot 10^{+7}$ spores par ml à l'aide du serum physiologique glyceriné au demi et saponiné (concentration finale en saponine -1 p 1 000).

L'ajustement du titre, la quantité d'eau physiologique, de glycérine ainsi que la quantité de saponine sont définis selon la formule ci-dessous :

$$N_1 V_1 = N_2 V_2 \quad V_2 = \frac{N_1 V_1}{N_2}$$

$$Q_{\text{saponine}} = \frac{\text{Quantité de la souche-mère} + \text{quantité totale de glycérine et d'eau physio}}{1000} \times 1$$

Q_{saponine} = quantité de saponine

Ces quantités obtenues sont aussitôt mélangées et ajoutées à la suspension mère glycérinée

6. Contrôle du vaccin

- pureté gram, état frais
- titre denombrement des colonies sur gelose

V. REFARCTION

Elle est faite à l'aide d'une seringue automatique dans les flacons de 20 ml qui seront par la suite capsulés.

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VII. MODE D'EMPLOI DU VACCIN

1 ml par voie sous-cutanée

VIII. CONSERVATION

- à +4°C à l'abri de la lumière

IX. IMMUNITE

Installation au bout de 5 jours

Durée 1 an.

(1)

PROGRAMMATION DE LA PRODUCTION DU VACCIN
ANTI-PASTEURELLIQUE OVINS - CAPRINS.
NOM DE CODE : PASTOVIN

-o-o-o-o-o-o-o-o-

PROTOCOLE :

- JO Montage, stérilisation des ballons et filtres
Préparation du milieu de sterne
- JO+1 clarification du milieu de sterne (EK-filtre-Seitz)
. stérilisation du milieu de sterne clarifié (EKS filtre Seitz)
. incubation à 37°C du milieu de sterne stérilisé durant 48 he
- JO+2 Repiquage de la souche mère pasteurella multocida A sur TS
- JO+3 Ensemencement de l'inoculum (pasteurella multocida A)
stérilisation du fermenteur
- JO+4 Mise en marche du fermenteur
mise au vieillissement des cultures de pasteurella multocida A à
l'étuve (37°C)
- JO+6 Repiquage de la souche mère pasteurella multocida D sur TSA
- JO+7 Ensemencement de l'inoculum stérilisation du fermenteur
- JO+8 Mise en marche du fermenteur, préparation de l'alun de potasse
mise au vieillissement des cultures de pasteurella multocida D à 37°C
- JO+9 Stérilisation des matériels de sortie du vaccin; flacons de 500
eau physiologique alun de potasse
- JO+10 Repartition du vaccin
. conditionnement
. test de pureté et de stérilité.

VACCIN CONTRE LA PASTEURELLOSEDES OVINS-CAPRINSNom de code : PastovinI SOUCHE

Pasteurella multocida type A

Pasteurella multocida type D

II VACCIN

Culture dense de pasteurella multocida A et D, formolée vieillie à 37°C et adjuvée par l'alun de potasse.

III PREPARATION DES INOCULUMS : Trypticase soy broth - TSB

- 1° préparer et stériliser le TBS dans deux erlenmeyers de 500, servant d'inoculum.
- 2° ensemencer les boîtes de petri (TSA) enrichi de serum de cheval incuber à 37°C durant 18 - 24 heures.
- 3° contrôler la pureté des colonies (état frais gram) ensemencer les inoculums par pasteurella multocida A et D et mettre à l'étuve (37°C) durant 24 heures.
- 4° Contrôler les caractères des inoculums état frais, gram.

IV PREPARATION DU VACCIN :1° Préparation du milieu de sterne composition

| | |
|--|-------------|
| Extrait de viande (Difco ou Leibig ou Merieux) | 50 grs |
| Bacto peptone | 100 grs |
| Nacl | 50 grs |
| Digestat papaïnique de pancréas de boeuf | 1250 ml |
| glucose | 20 grs |
| Lactate de sodium à 60% | 33 ml |
| phosphate monosodique NaH ₂ PO ₄ | 30 grs |
| Eau distillée q.s. p..... | 8,750 litre |
| pH = 7,4 - 7,6 | |

Obtention du digestat de pancréas:

pancréas 1,8 kgs arrêter la température à 50°
 Eau distillée 6 000 ml

Ajouter 6grs de papaïne Merck en suspension dans 150 ml d'eau distillée.

Chauffer doucement en faisant monter progressivement la température de 50 à 70°C. Dès que la digestion est terminée porter la température à 85°C pendant 1 minute. Ensuite filtrer à chaud à travers les membranes clarifiantes EK (filtre Seitz). Le digestat rentre dans la préparation du milieu de Sterne à raison de 1250ml pour 8 750 ml de milieu.

Le milieu de sterne ainsi préparé et clarifié sur membrane clarifiante EK est ensuite stérilisé sur membrane EKS (filtre Seitz).

2° contrôle de stérilité des ballons de production contenant le milieu de Sterne incubation à 37°C durant 48 heures.

3° Stérilisation du fermenteur :

3.1. remplir le fermenteur d'eau distillée

3.2. mettre l'interrupteur principal (Main Power Switch) sur ON

3.3. placer le bouton d'agitation (Agitation Swratch) en position ON, et tourner le bouton de vitesse d'agitation (Agitation contrôl) dans le sens des aiguilles d'une montre jusqu'à ce que 400tr/mn soient indiquée par le cadran Agitation Tachometer.

3.4. Fermer les valves suivantes si elles sont ouvertes;

Air Floumeter, Main sparger, Vessel Exhaust

3.5. Ouvrir la valve exhaust condensate si elle est fermée

3.6. fixer la température de stérilisation (121°C) lire sur digital en maintenant l'inverseur à bascule et en tournant la molette dans le sens des aiguilles d'une montre.

3.7. placer en position -stérilize -l'interrupteur sterilization OFF Grouth control.

Attention : quand la pression du fermenteur atteint 5 Psig il faut ouvrir les valves suivantes pendant 5mn avant de les refermer: Sparger Steam, Main Sparger, Drain Steam.

Le temps minimum de stérilisation à 121°C est de 30 minutes

3.8 Ouvrir pendant 30mn. Les valves suivantes: Inlet filter condensate valve, Sparger Steam valve.

3.9 Stériliser pendant 5 mn tous les ports d'entrée après les avoir ouvert sur un tour

Ensuite on procède à un refroidissement rapide du fermenteur après avoir fini la stérilisation.

3.10. Refroidissement rapide du fermenteur

3.10.1. fermer les valves suivantes: Exhaust condensate, Sparger Steam, Inlet filter condensate.

3.10.2. Placer sur off la manette Stériliz/off/Growth control

3.10.3. Ouvrir les valves suivantes : Main sparger, rapide cooling, Main water.

3.10.4 Regler la molette du contrôl de température sur la température d'incubation de 37°C (en maintenant l'inverseur à bascule et en tournant la molette dans le sens contraire des aiguilles d'une montre) placer sur la position Growth l'interrupteur de contrôle.

3.10.5. Lorsque la température n'est plus au dessus de la température d'incubation (37°C) que de quelques degrés, fermer rapidement la valve de refroidissement (valve rapid cooling)

3.10.6. Ouvrir la valve vessel exhaust et regler la pression de retour et le debit d'air.

Remarque : Si durant l'incubation, la température dépasse ou n'atteint pas la température d'incubation désirée, agir sur la valve cooling contrôl (1T/34). Après la fin des étapes 3.10.4 et 3.10.5 le fermenteur est sur le mode de température contrôlée et est prêt pour être utilisé.

3.10.7. On déverse le contenu (Eau distillée) du fermenteur dans un ballon de 10 litres par l'orifice d'échantillonnage après avoir ouvert les valves:

Main sparger et vessel Exhaust; agir sur le régulateur de retour de pression pour maintenir une pression positive dans le fermenteur ; ajuster le floumèter et regler la pression. Cette procedure permet l'aération du fermenteur.

- 3.11. Introduire l'inoculum, ensuite le milieu de Sterne/l'antimousse pour cela proceder comme suit :
 - 3.11.1 Fermer le Main Sparger valve puis reduire la pression à Opsig en ouvrant le Back pressure regulator.
 - 3.11.2 introduire aseptiquement l'inoculum après avoir ouvert le port d'inoculation.
 - 3.11.3 aerer normalement en utilisant le Main Sparger valve et le Back Pressure regulator.
 - 3.11.4 Avec la pression reduite à 0 Psig introduire le milieu de Sterne grace à la pompe automatique.
- 3.12. Ajuster la température à 37°C, mettre l'agitation (Agitation Switch on) en marche, ajuster la rotation à 400 Tr/mn.

La culture a ainsi débuté : à travers un écran illuminé on controlera visuellement la densité des pasteurelles durant la culture afin de déterminer le moment où il faudra changer le milieu de Sterne après avoir vidanger le contenu du fermenteur (culture prête) dans un ballon de 10 litres contenant au préalable 50ml de formol à 37% d'aldehyde formique.

N.B. vidanger jusqu'à ce qu'il ne reste qu'une hauteur de 60cm de culture dans le fermenteur. En ce moment fermer la pince.
- 3.13. Pendant le vidange de la culture il faut recueillir un échantillon de culture à l'orifice d'échantillonage (Sampling Port) en vue de contrôler la pureté de la culture obtenue.
- 4) Arrêt total du fermenteur

pour arrêter complètement le fermenteur après toutes les opérations.

 - 4.1 Reduire la pression à 0 psig en fermant le Main Sparger valve et le flouwmeter valve si elles sont ouvertes. Ouvrir le black pressure.
 - 4.2. Fermer toutes les valves du tableau de bord frontal placer toutes les manettes sur la position Off tourner tous les boutons de contrôle dans le sens contra des aiguilles d'une montre jusqu'à zéro (0).

- 4.3. Si l'appareil doit demeurer longtemps sans être utilisé, fermer aussi les valves externes (eau, air, vapeur).
5. Toutes les cultures formolées sont mises au vieillissement (37°C) durant 48 - 72 heures.
9. Après le vieillissement les cultures formolées pasteurisées multocida A et Pasteurella multocida D sont mélangées ensuite diluées avec l'eau physiologique stérile selon leur densité opacimétrique. On prendra comme référence les tubes standard de Brown (ou l'échelle de Mac Farland) Cependant la dilution est faite de manière à obtenir la densité du tube N°8 de brown. Les cultures A et D sont mélangées à parties égales.
10. Additionner l'alun de potasse (concentration finale 1 p. 100).
11. Formoler les cultures diluées et adjuvée afin d'obtenir une concentration finale de 4-5 p. 1000.
12. Répartition du vaccin
Le vaccin sous agitation magnétique est repartit dans les ballons de Wolf à l'aide d'une pompe automatique, ensuite distribué dans les flacons de 500ml.

V. Contrôle du vaccin

- pureté état frais, Gram
- stérilité : ensemencement sur TSA, gelose Sabouraud, gelose profonde, gelose anaérobie, VF milieu de sternac, TSB, FTM, bouillon ordinaire.

Les autres tests (inocuité, efficacité etc...) s'effectueront dans une section contrôle de qualité avant la livraison du vaccin.

VI. Mode d'Emploi

2ml en sous-cutané par animal

VII. Conservation

- à + 4°C la durée de conservation est de 2 ans.
- à la température ambiante cette durée n'excède pas 6 mois

VIII. Immunité

Elle dure 6 mois.

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PROGRAMMATION DE LA PRODUCTION DU VACCIN
ANTI-PASTEURELLIQUE - BOVINS
NOM DE CODE : PASTOBOV.

Protocole

- Jo Montage , stérilisation des ballons et filtres
préparation du milieu de sterne
- Jo+1 .Clarification du milieu de sterne (EK Filtre Seitz)
.Stérilisation du milieu de sterne clarifié (EKS filtre Seitz)
.inoculation à37°c du milieu de sterne stérilisé durant
48 heures.
- Jo+2 Repiquage de la souche mère pasteurella multocida E sur TSA
- JO+3 Ensemencement de l'inoculation, stérilisation du fermenteur
- Jo+4 Mise en marche du fermenteur inoculation, récolte
mise au viciilissement 48-72 heures à l'étuve (37°c)
préparation de l'alun de potassium
Stérilisation des matériels de sortie du vaccin flacons de
500ml eau physiologique, alun de potasse.
- Jo+7 Repartition du vaccin
.Conditionnement
.test de pureté, stérilité.

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VACCIN CONTRE LA PASTEURELLOSE BOVINENON DE CODE : PASTOBOVI SOUCHE

Pasteurella multocida type E

II VACCIN

Culture dense de pasteurella multocida type E, formolée, vieillie à 37° et adjuvée par l'alun de potassium.

III Preparation de l'inoculum : Trypticase soy broth- TSB

1. preparer et steriliser le TSB dans un erlenneyer de 500 ml servant d'inoculum
2. ensemencer les boites de Petri (TSA enrichi de serum de cheval) incuber à 37°c durant 18- 24 heures
3. controler la purité des colonies (état frais, gram) ensemencer l'inoculum et mettre à l'etuve (37°c) durant 24 heures
4. contrôler les caractères de l'inoculum etat frais, gram

IV. PREPARATION DU VACCIN

1. preparation du milieu de culture-milieu de Sterne
composition

| | | |
|--|-------|--------|
| extrait de viande (DIFCO ou Leibig ou bien Merieux)..... | 50 | grs |
| Bacto-peptone (DIFCO)..... | 100 | grs |
| Nacl..... | 50 | grs |
| Digestat papainique de pancréas de boeuf..... | 1250 | ml |
| glucose..... | 20 | grs |
| lactate de sodium à 60%..... | 33 | ml |
| phosphate monosodique (NaH_2Po_4)..... | 30 | grs |
| eau distillée, q.s.p..... | 8,750 | litres |

PH = 7,4 - 7,6

Obtention du digestat de pancreas :

| | | | |
|---------------|---------|---|------------------------------|
| pancreas | 1,8 kg |] | porter la température à 50°c |
| eau distillée | 6000 ml |] | |

Ajouter 6 grs de papaine Merck en suspension dans 150 ml d'eau distillée.

Chauffer doucement en faisant monter progressivement la température de 50 à 70°c. Dès que la digestion est terminée, porter la température à 85°c pendant 1 mn. Ensuite filtrer à chaud à travers les membranes clarifiantes EK (filtre Seitz). Le digestat rentre dans la préparation du milieu de Sterne à raison de 1250 ml pour 8,750 litres de milieu.

2. Contrôle de sterilité des ballons de production contenant le milieu de Sterne incubation à 37°C durant 48 heures.
3. Sterilisation du fermenteur :
 - 3.1. remplir le fermenteur d'eau distillée à l'aide d'une pompe automatique
 - 3.2. mettre l'interrupteur principal (Main Power Switch) sur ON
 - 3.3. placer le bouton d'agitation (Agitation Switch) en position ON, tourner le bouton de vitesse d'agitation (Agitation control) dans le sens des aiguilles d'une montre jusqu'à ce que 400 tr/mm soient indiqués par le cadran Agitation Tachometer
 - 3.4. fermer les valves suivantes si elles sont ouvertes :
Air Flowmeter, Main Sparger, Vessel Exhaust
 - 3.5. Ouvrir la valve Exhaust condensate si elle est fermée :
 - 3.6. fixer la température de sterilisation (121°C) lire sur digital en maintenant l'inverseur à bascule et en tournant la molette dans le sens des aiguilles d'une montre
 - 3.7. placer en position sterilize l'interrupteur sterilize/OFF/GROWTH control.
attention : Quand la pression du fermenteur atteint 5PSIG , il faut ouvrir les valves suivantes pendant 5 minutes, avant de les refermer :
Sparger Steam, Main Sparger, Drain Steam.
Le temps minumum de sterilisation à 121°C est de 30 minutes
 - 3.8. Ouvrir pendant 30 minutes les valves suivantes :
Inlet filter condensate valve, Sparger Steam valve
 - 3.9. Steriliser pendant 5 minutes tous les ports d'entrée après les avoir ouvert sur un tour. Ensuite on procède à un refroidissement rapide du fermenteur après avoir fini la sterilisation
 - 3.10. refroidissement rapide du fermenteur :
 - 3.10.1. fermer les valves suivantes : Exhaust condensate
Sparger Steam, Inlet filter condensate
 - 3.10.2. placer sur OFF la manette Sterilize/OFF/ GROWTH control
 - 3.10.3. Ouvrir les valves suivantes : Main Sparger, Rapid cooling, Main water
 - 3.10.4. Regler la molette du control de température sur la température d'incubation de 37° (en maintenant l'inverseur à Bascule et en tournant la molette dans le sens contraire des aiguilles d'une montre)
Placer sur le position GR WTH l'interrupteur de contrôle
 - 3.10.5. Laisser le fermenteur se refroidir au dessus de la température d'incubation (+, -) que de quelques degrés, fermer rapidement la valve de refroidissement (valve Rapid cooling).
 - 3.10.6. Ouvrir la valve vessel Exhaust et régler la pression de retour et le débit d'air.

Remarque : Si durant l'incubation, la température dépasse ou n'atteint pas la température d'incubation désirée, agir sur la valve cooling control (1T/314). Après la fin des étapes 3.10.4. et 3.10.5. le fermenteur est sur le mode de température contrôlée et est prêt pour être utilisé.

- 3.10.7. On déverse le contenu (eau distillée) du fermenteur dans un ballon de 10 litres par l'orifice d'échantillonnage après avoir ouvert les valves : main Sparger et vessel Exhaust; agir sur le régulateur de retour de pression pour maintenir une pression positive. Cette procédure permet l'aération du fermenteur.
- 3.11. Pour introduire l'inoculum, introduire d'abord le milieu de Sterne et l'antimouss ensuite procéder comme suit :
- 3.11.1. Fermer le Main Sparger valve puis réduire la pression à OPSIG en ouvrant le Back pressure Regulator
- 3.11.2. introduire aseptiquement l'inoculum (*Pasteurella multocida A*) après avoir ouvert le port d'inoculation .
- 3.11.3. Aérer normalement en utilisant le Main Sparger valve et le Back Pressure Regulator
- 3.11.4. Avec la pression réduite à OPSIG introduire le milieu de Sterne grâce à la pompe automatique.
- 3.12. Ajuster la température à 37°C, mettre l'agitation (Agitation Switchon) en marche, ajuster la rotation à 400 tr/mm.
La culture à ainsi débuté : à travers un écran illuminé on contrôlera visuellement la densité des pasteurelles afin de déterminer le moment où il faudra introduire de nouveau le milieu de Sterne; et cela après avoir effectué le vidange du fermenteur dans un ballon de 10 litres contenant 50 ml de formol (37% d'aldehyde formique)
N.B. faire le vidange jusqu'à ce qu'il ne reste plus qu'une hauteur de 60 cm de culture dans le fermenteur. En ce moment fermer la pince située sur le raccord lié à l'orifice d'échantillonnage.
- 3.13. Pendant le vidange de la culture on peut recueillir un échantillon de culture par l'orifice d'échantillonnage (sampling Port) en vue de contrôler la pureté de la culture.
4. On procède à la stérilisation du fermenteur selon le point 3
5. ensuite on introduit l'inoculum *Pasteurella multocida D* (voir point 3.11.)
.....(le milieu de culture et à déterminer (voir point 3.11.))
7. Après l'incubation de toutes les cultures on effectue le vidange (voir point 3.10)
4. Arrêt total du fermenteur.
Pour arrêter complètement le fermenteur après toutes les opérations
- 4.1. Réduire la pression à 0 PSIG en fermant le Main Sparger Valve et le flowmeter

valve si elles sont ouvertes. Ouvrir le Back Pressure

- 4.2. Fermer toutes les valves du tableau de bord frontal
placer toutes les manettes sur la position OFF.
Tourner tous les boutons de control dans le sens contraire des aiguilles d'une montre jusqu'à Zero (0)
- 4.3. Si l'appareil doit demeurer longtemps sans être utilisé, fermer aussi les valves externes (eau, air, vapeur)
5. Toutes les cultures formolées sont mises au vieillissement (37°C) durant 48 - 72 heures.
6. Après le vieillissement les cultures sont diluées avec l'eau physiologique stérile selon leur densité opacimétrique. On prendra comme référence les tubes standard de Brown où l'échelle de Mac Farland.
7. Ajouter l'alun de potasse (concentration finale 1 p. 100)
8. Formoler la culture diluée et adjuvée avec du formol afin d'obtenir une concentration finale de 4-5 p. 100;
9. Répartition du vaccin fini

Le vaccin sous agitation est réparti dans les ballons de Wolf à l'aide d'une pompe automatique. Ensuite il est distribué dans les flacons de 500 ml.

V Contrôle du vaccin

- pureté état frais, gram
- stérilité ensemencement sur TSA, gélose ordinaire, gélose profonde, gélose anaérobie, gélose salouraud, TSA, bouillon ordinaire, FTM, VF, milieu de Sterne.

VI Mode d'emploi

2 ml par animal par la voie sous-cutanée

VII Conservation

- à + 4°C la durée de conservation est de 2 ans environ
- à la température ambiante 6 mois

VIII immunité conférée par le vaccin

Elle n'excède pas 6 mois

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METHODES DE PRODUCTION ET DE CONTROLE
DES VACCINS AVIAIRES

PAR

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3 DECEMBRE 1988

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/-) VANT PROPOS

- L'industrie avicole dans la plupart des pays en voie de développement connaît une croissance rapide. L'aviculture non seulement permet de couvrir en partie le déficit protéique d'origine animale chez les humains mais aussi fournit l'emploi et la liquidité aux familles.
C'est ainsi que plusieurs fermiers convertissent leur arrière cours en entreprises avicoles commerciales.
- Pendant leur vie économique, disons de 18 mois, environ 10-12 doses de vaccins de différentes sortes sont nécessaires. La production des vaccins devient un imperatif pour limiter les pertes causées par diverses maladies et assurer un développement harmonieux de l'industrie avicole.
Les vaccins sont produits à partir d'oeufs de poules élevées dans un environnement stérile. La technologie d'élevage de volailles exemptes de germes spécifiques (SPF : specific pathogen free) n'est pas encore à la portée des pays en voie de développement à cause de facteurs divers. Cependant, pour le démarrage d'une telle ferme on doit disposer d'une petite ferme semi SPF d'individus non vaccinés. Le cheptel devra être exempt de germes transmissibles aux oeufs.
- Les vaccins importés non seulement sont chers et nécessitent des devises mais aussi ont des problèmes inhérents à la conservation, à cause de la durée de transport et du temps de souffrance pendant le dédouanement.
Au-delà de ces considérations, ils ne sont pas conditionnés pour satisfaire les besoins des petits fermiers de nos pays. En plus les méthodes d'administration ne sont convenables qu'à l'échelle de grandes entreprises, vaccination par aerosol etc...
- La préparation locale des vaccins aviaires est par conséquent vitale pour l'autosuffisance et l'autodépendance. Les méthodes de production de vaccins aviaires et le contrôle de qualité ont pour but de fournir toutes les informations de production et d'utilisation des vaccins les plus récents dans l'industrie avicole.

VACCIN CONTRE LA MALADIE DE NEWCASTLE

CONSIDERATIONS GENERALES

1. Parmi toutes les maladies aviaires, la maladie de Newcastle continue à hypothéquer le plus la vie des volailles dans les pays en voie de développement. Elle a été éradiquée en Angleterre et est sous contrôle en Europe et en Amérique du Nord.
2. La maladie atteint les oiseaux susceptibles de tous les groupes d'âge et la mortalité pourrait s'élever à 100 %. Elle se propage par contact direct ou indirect et souvent en faveur des vents.
3. Les souches virulentes sont gardées en circulation pour des oiseaux convalescents et des porteurs immunisés tels que les poules de race locale, les canards et les oiseaux sauvages.
4. Du point de vue clinique la maladie présente 4 formes :
 - a. la forme viscerotropique ou enterotropique de Doyle.
 - b. la forme neurotropique de Beach
 - c. la forme respiratoire de Beaudette
 - d. la forme respiratoire bénigne de Hitchner
5. Au début des années 70 en Amérique du Nord et en Europe, la forme Vélogenique viscerotropique de la maladie de Newcastle étant pandémique. Elle ressemblerait à la forme de Doyle caractérisée par un œdème faciol, symptôme spécial
6. La maladie de Doyle est rencontrée dans les pays en voie de développement. Une étude récente des souches de l'Afrique de l'Ouest révélait que celles-ci n'ont pas changé depuis plusieurs décades.
7. Au Laboratoire les souches virales sont classifiées en souches :
 - a) Vélogenique ou fortement virulente
 - b) Mésogénique ou moins virulente
 - c) Lentogénique ou avirulentepar leur propriété pathogénique lorsqu'inoculées par voie intramusculaire (IM) aux poussins âgés de 6 semaines.
par voie intracérébrale aux poussins d'un jour et par délai moyen de mort (DM) aux embryons de poulet en développement (en croissance).

8. Antigéniquement les souches diffèrent lorsque testés par les anticorps monoclonaux, mais toutes les souches vaccinales protègent contre toutes les souches pathogéniques connues.
9. Il existe 3 types de vaccins disponibles sur le marché
 - a) Vaccins lentogeniques : Bl, lasota, F, Queens land V4 etc;
 - b) Vaccins mésogéniques : Komarov, Mukteswar, Roakin, Bankowski.
 - c) Vaccins inactivés à émulsion huileuse ou adjuvés d'hydroxyde d'alluminium : GB Texas, lasota, Ulster 2C
10. Les vaccins à souches Bankowski ou KT (Komarov en culture tissulaire) sont mésogéniques et ne peuvent être administrés qu'aux individus de plus de 8 semaines. Ils ont un titre moins élevé par rapport au vaccins préparés en œufs et sont plus sensibles aux anticorps existants (anticorps maternel ou vaccinal).
11. Les vaccins à émulsion huileuse (EH) peuvent être administrés aux oiseaux âgés d'un jour mais nécessitent un rappel à 3 semaines et juste avant l'entrée en ponte. Ces vaccins ne remplacent pas les vaccins vivants mais les complètent. Ils sont administrés par voie intramusculaire seulement et sont mieux adaptés pour les vaccinations de rappel, les vaccinations d'urgence et la vaccination des reproducteurs. Une meilleure réponse immunitaire est obtenue par combinaison de vaccin vivant et de vaccin à émulsion huileuse donnée aux poussins d'un jour.
12. Les vaccins mésogéniques vivants devront également être administrés par voie IM ou SC (sous-cutanée). Ils n'échappent pas à la recrudescence. Cependant, ils confèrent une immunité uniforme et solide avec une dose minimale de 10^5 D1050 de vir par sujet.
13. Parmi les vaccins lentogéniques les souches Hitchner Bl et lasota sont utilisées dans le monde entier spécialement pour les poussins. Elles cultivent très aisement dans l'œuf de poulet et donnent un liquide amnio-allantoïdien (LAA) d'un titre élevé convenable pour les vaccinations de masse dans l'eau de boisson, par aerosol, par instillation dans l'œil, dans les narines ou absorption par le bec. Elles ne poussent pas facilement en culture cellulaire. Par comparaison aux souches mésogéniques les vaccins à souches lentogéniques requièrent un titre situé entre $10^{6.5}$ et 10^7 D1050 par oiseau.
14. Il existe une variété de programmes de vaccination adaptés aux différentes situations de terrain. Cependant la suggestion suivante peut être faite : administrer
 - aux poussins d'un jour à l'éclosoir le vaccin HBl
 - à l'âge de 3 semaines le vaccin lasota suivi d'un rappel
 - à l'âge de 12 semaines avec la souche Komarov ou un vaccin à EH.La souche lasota vu sa nature dispersive est très bien adaptée pour les vaccinations d'urgence par exemple lorsqu'un foyer est déclaré dans les fermes environnantes.

15. Un programme de vaccination bien élaboré et exécuté reduit l'incidence de la maladie clinique sur la ferme sinon dans une localité. Ceci n'empêche pas l'infection. Par contre l'hygiène et l'assainissement préviennent à un degré très élevé l'introduction de la maladie dans une ferme, par conséquent ils devraient être associés à la vaccination.

Il n'y a pas de substituts à l'hygiène, à l'assainissement et aux mesures de quarantaine. Toutes ces mesures doivent être rigoureuses.

PRODUCTION DE VACCIN CONTRE LA MALADIE DE NEWCASTLE
DE SOUCHES Bl, LA SOTA ET KOMAROV

1. Lorsqu'on doit produire plus d'un vaccin aviaire, le travail devra être organisé de manière à produire un type de vaccin et le lyophiliser sans conflit d'opérations. Cela permet le nettoyage et la désinfection des locaux pour l'étape suivante.
2. Le vaccin Bl est produit à partir d'oeufs embryonnés de 9-10 jours mais seront incubés pendant 5 jours avant la récolte du liquide amnio-allantoidien (LAA) alors que les vaccins à souche lasota et Komarov utilisent des oeufs embryonnés de 10 jours pour une durée d'incubation de 3 et 2 jours respectivement à 37°C. Les oeufs sont ensuite refroidis avant la récolte afin d'éviter la saignée des embryons.
3. Le titre de la semence de virus doit être vérifié tous les six mois et une nouvelle semence préparée si cela s'avère nécessaire à partir de la banque. Le titre doit être supérieur à $10^{9,5}$ par ml et le titre hemagglutinant (HA) de 1 : 128 ou plus.
4. Pour éviter les contaminations, les cabines d'inoculation et de récolte seront fumigées avec le formol et le permanganate de potassium ou désinfectées avec une solution de phenol à 1 % dans l'eau de robinet pendant 5 - 10 minutes plusieurs heures avant leur utilisation.
5. Les vêtements stériles de protection, les bonnets, les masques et les gants seront portés par tous ceux qui travailleront dans les cabines pour l'inoculation ou la récolte. Les mains seront lavées proprement avec du savon et une brosse sera utilisée pour nettoyer les ongles. Les mains sechées seront désinfectées à l'alcool phénique (1 % de phenol dans l'alcool à 70°C).
6. Pour le mirage, ne sortir que quelques alvéoles pour un temps bien limité afin de préserver la viabilité des embryons.
7. Les oeufs doivent être mirés après 7 jours d'incubation et juste avant l'inoculation. Les oeufs clairs doivent être éliminés aussi bien que ceux ayant des embryons morts. Les embryons en bon état de santé présentent une vascularisation proéminente et sont eux-mêmes très actifs pendant le mirage. L'embryon vivant apparaît comme un point sombre correspondant à la tête ou à l'œil avec une ombre diffuse, la membrane chorioallantoïdienne entourant l'embryon. Avec, l'âge la chambre à air, l'embryon et la membrane chorioallantoïdienne augmente en volume.
Les vaisseaux d'un embryon mort sont interrompus et peu visibles. Par occasion, des rayures épaisses, rouges, provoquées par une saignée sont visibles. De tels oeufs doivent également être écartés puisque les embryons mourront avant de pouvoir supporter la croissance du virus.

8. Répéter au crayon la base de la chambre à air.
Marquer un point à 4 mm environ au dessus de la chambre à air.
9. Désinfecter la région de la chambre à air à l'alcool iodé.
Laisser sécher pour éviter l'inactivation du virus.
10. Perforer la coquille au foret. Le trou devra être suffisamment grand pour permettre le passage de l'aiguille pour l'inoculation et suffisamment petit pour être facilement obturé (1 mm).
De la même manière perforer un trou au centre de la chambre à air et ceci avant le trou latéral.
11. Utiliser une aiguille d'un calibre fin et pénétrer de 10 à 14 mm verticalement.
Injecter 0,1 à 0,2 ml de l'inoculum à chaque oeuf (Dil 10⁻³ à 10⁻⁵ de la semence).
12. Obturer les trous avec un mélange cire-vaseline liquefié par la chaleur. Incuber à 37° C dans un incubateur statique bien ventilé à hygrométrie située entre 60 et 65 %.
13. Après incubation, les oeufs sont mirés et gardés dans une chambre froide toute une nuit. Apprêter environ 7 flacons par oeuf pour la lyophilisation si chaque flacon doit recevoir 2 ml de vaccin.
14. Les oeufs sont désinfectés à l'alcool iodé dans les limites de la chambre à air et légèrement en dessous. Laisser secher et ouvrir l'oeuf selon le contour de la chambre à air à l'aide de ciseaux.
Faire un test rapide d'hémagglutination (HA) et rejeter tout oeuf qui n'aura pas été positif.
A l'aide d'une cuillère à café déprimer l'embryon et aspirer le LAA avec une pipette pasteur elle même au bout d'un raccord rattaché à un ballon de collecte. Prendre soins de ne point aspirer ni le sang ni le vitellus ni l'albumen car ceux-ci réduisent le titre du vaccin. Tout oeuf suspect est à écarter.
15. Aucune nécessité de filtrer ou de centrifuger le vaccin si le mirage et la récolte sont faits avec rigueur.
16. Ajouter un volume égal de stabilisateur au vaccin et procéder au contrôle bactériologique sur gelose au sang, gelose au thioglycollate et gelose sabouraud. On peut aussi ajouter des antibiotiques au taux de 1 M d'unités de pénicilline et 1 g de streptomycine à 1 litre de vaccin. Repartir à raison de 2 ml/flacon.
17. Après lyophilisation le vaccin sera encore soumis à un contrôle bactériologique. Le titrage sera fait pour déterminer son point final ou DIO₅₀ par dose (ou par ml ce qui est de 100 doses). Le test HA est simplement estimatif.

18. Determination du titre (DIO_{50})

Après reconstitution du vaccin (10°) faire des dilutions décimales en ajoutant 0,5 ml de la suspension vaccinale à 4,5 ml de PBS et cela jusqu'à la dilution 10^{-10} . Utiliser les dilutions 10^{-6} - 10^{-10} pour inoculer 4-6 oeufs par dilution à raison de 0,1 ml par voie intra allantoidienne.

Après une période d'incubation variant de 2-5 jours selon les souches les oeufs sont gardés au froid pour une nuit, un test HA est pratiqué, utilisant le contenu d'une anse prélevé sur chaque oeuf et mélangé avec une goutte d'une suspension à 5 % de globules rouges sur une plaque en porcelaine; les résultats sont enregistrés (cas positifs et négatifs) et le titre calculé par la méthode cumulatine de Reed et Muench.

19. Dix oiseaux recevront chacun 10 doses pour le test d'innocuité. Ils devront rester en bonne santé. Pour les souches HB₁ et Lasota des poussins de 3 semaines seront utilisés et pour la souche Komarov des poussins de 6-8 semaines feront l'objet du test.

20. Le test d'efficacité utilisera 10 oiseaux qui seront vaccinés et éprouvés 3 semaines plus tard alors que 10 autres serviront de témoins. Le virus d'épreuve est la souche Herts sous forme de LAA de 24-48 heures dilué au 1/10 dont la dose par sujet est de 0,1 ml. Ceci équivaut approximativement à 10^4 - 10^6 DLE₅₀ (Dose lethale embryonique).

Tous les individus vaccinés resteront indemnes et environ 80 % des témoins mourront pour que le test soit concluant.

B. LYOPHILIZER WITH ACCESSORIES AND PARTS

1. SUBLIMATOR, 6 product shelves; with stoppering device capable to stoppering several serum bottle sizes in a single run; with tray area of at least 3.1 square meters; condenser ice capacity of at least 35 Kg in 24 hours; shelf clearance of at least 65 mm.; capable of providing shelf temperatures in the range of -50 C to +65 C; condenser temperatures with maximum low of at least -62 C; pump down in dry chamber to at least 50 microns in 20 minutes; ELECTRIC SERVICE TO BE COMPATIBLE WITH LOCAL AVAILABILITY OF 380 V/3 PHASE/50 CYCLE. 208/230 V/3 PHASE/60 CYCLE IS NOT COMPATIBLE WITH LOCAL SERVICE; 18/8 stainless steel construction in at least shelves, stoppering lead screws, chamber walls, condensers, feed throughs, vacuum ports, and heat transfer manifolds; controls to at least 5 product probes with capability of individual temperature readouts, temperature gauge, vacuum gauge, shelf temperature controller with digital setting readout, condenser temperature controller with digital setting readout, plus required controls for freezing, condenser operation, vacuum, shelf heat, defrosting, and stoppering

One each, CIF, Bamako, Mali.....\$95,000.00

VIRTIS MODEL 101-SRC-6 or equal

(Due to specific training of operators, the extended familiarity of operators with Virtis machines, the difficulty in obtaining and installing spare parts from multiple manufacturers, and the remoteness of the site of use, it is requested that the strongest consideration be given to purchase of an additional Virtis machine to replace Virtis machines already in use and nearing end of useful life)

2. SPARE PARTS FOR ITEM 1.: to include those deemed to be required for uninterrupted operation but to include at least the following:

- a. vacuum pump
- b. compressor
- c. hoses
- d. door gaskets
- e. circulation heater
- f. R-502 refrigerant
- g. heat transfer fluid

Five Year Supply, CIF, Bamako, Mali.....\$11,200.00

3. RECORDER; 6 point temperature and vacuum point recording; with sufficient temperature and vacuum recording chart paper and recorder pen ink to last at least 180 days of operation. Recorder must be fully compatible with item 1

One each, CIF, Bamako, Mali.....\$8,700.00

L & N Model 250 or equal

4. STAINLESS STEEL TRAYS WITH COVERS; standard design, must be fully compatible with item 1; with hold down design to insure maximum contact with shelf surface; sample bottles will be provided by user upon request.

Twenty-four each, CIF, Bamako, Mali.....\$5,300.00

5. VACUUM PUMP OIL; fully compatible with item 1.; stable up to 50 C; constant viscosity between 15 C and 50 C; in one gallon containers.

Six Containers, CIF, Bamako, Mali.....\$225.00

VIRTIS No. 3810-0022 or equal

6. CHARGING PUMP; for vacuum pump oil; to fit containers in item 5; complete with hose.

One each, CIF, Bamako, Mali.....\$275.00

VIRTIS No. 6270-1375 or equal

7. HIGH VACUUM GREASE; capable of retaining consistency from -40 C to +260 C and at 0.01 millitorr

Three Containers, CIF, Bamako, Mali.....\$100.00

DOW CORNING HIGH VACUUM GREASE or equal

or, alternatively

1. SUBLIMATOR, 7 product shelves; with stoppering device capable to stoppering several serum bottle sizes in a single run; with tray area of at least 7.8 square meters; condenser ice capacity of at least 120 Kg in 24 hours; shelf clearance of at least 93.4 mm.; capable of providing shelf temperatures in the range of -50 C to +65 C; condenser temperatures with maximum low of at least -62 C; pump down in dry chamber to at least 50 microns in 20 minutes; ELECTRIC SERVICE TO BE COMPATIBLE WITH LOCAL AVAILABILITY OF 380 V/3 PHASE/50 CYCLE. 208/230 V/3 PHASE/60 CYCLE IS NOT COMPATIBLE WITH LOCAL SERVICE; 18/8 stainless steel construction in at least shelves, stoppering lead screws, chamber walls, condensers, feed throughs, vacuum ports, and heat transfer manifolds; controls to at least 5 product probes with capability of individual temperature readouts, temperature gauge, vacuum gauge, shelf temperature controller with digital setting readout, condenser temperature controller with digital setting readout, plus required controls for freezing, condenser operation, vacuum, shelf heat, defrosting, and stoppering

One each, CIF, Bamako, Mali.....\$203,700.00

VIRTIS MODEL 251-SRC-7 or equal

(Due to specific training of operators, the extended familiarity of operators with Virtis machines, the difficulty in obtaining and installing spare parts from multiple manufacturers, and the remoteness of the site of use, it is requested that the strongest consideration be given to purchase of an additional Virtis machine to replace Virtis machines already in use and nearing end of useful life)

2. SPARE PARTS FOR ITEM 1.: to include those deemed to be required for uninterrupted operation but to include at least the following:

- a. vacuum pump
- b. compressor
- c. door gaskets
- d. circulation heater
- e. R-502 refrigerant
- f. heat transfer fluid

Five Year Supply, CIF, Bamako, Mali.....\$15,500.00

3. RECORDER; 6 point temperature and vacuum point recording; with sufficient temperature and vacuum recording chart paper and recorder pen ink to last at least 180 days of operation. Recorder must be fully compatible with item 1

One each, CIF, Bamako, Mali.....\$8,700.00

L & N Model 250 or equal

4. STAINLESS STEEL TRAYS WITH COVERS; standard design, must be fully compatible with item 1; with hold down design to insure maximum contact with shelf surface; sample bottles will be provided by user upon request.

Fifty-six each, CIF, Bamako, Mali.....\$12,300.00

5. VACUUM PUMP OIL; fully compatible with item 1.; stable up to 50 C; constant viscosity between 15 C and 50 C; in one gallon containers.

Six Containers, CIF, Bamako, Mali.....\$225.00

VIRTIS No. 3810-0022 or equal

6. CHARGING PUMP; for vacuum pump oil; to fit containers in item 5; complete with hose.

One each, CIF, Bamako, Mali.....\$275.00

VIRTIS No. 6270-1375 or equal

7. HIGH VACUUM GREASE; capable of retaining consistency from -40 C to +260 C and at 0.01 millitorr

Three Containers, CIF, Bamako, Mali.....\$100.00

DOW CORNING HIGH VACUUM GREASE or equal

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C. STEAM GENERATORS

1. STEAM GENERATOR; electric, 60 kW; 6.0 developed boiler horsepower; steam capacity of least 94 kg/hour at 100 C; 380V/3 Ph/50 Hz operation; capable of producing at least 97% vapor quality; meeting UL, CSA, ASME codes; with automatic low-water heater shutoff; capable of reaching working temperature in 10 minutes or less; with automatic control for demand heat only; automatic flush and drain system; dimensions approximately 1124mmL x 559mmW x 832mmH (control box) x 673mmH (cabinet). NOTE: GENERATOR CANNOT BE MORE THAN ABOUT 16 METERS FROM STERILIZER.

Two each CIF Bamako, Mali.....\$12,000.00

AMSCO MODEL 60kW or equal

2. SPARE PARTS FOR ITEM 1.: to include those deemed to be required for uninterrupted operation but to include at least the following:

- a. electric heaters
- b. fuses
- c. contacts

Five Year Supply CIF Bamako, Mali.....\$3000.00

or, alternatively

1. STEAM GENERATOR, twin units; oil fired; using #2 fuel oil; 33 bhp each unit, output of at least 517 kg/hr per unit; oil consumption of approximately 37.5 lph per unit; for 380V/3ph/50Hz operation, controls to operate on 220V/1Ph/50Hz; with water needs of approximately 658 lph; complete with alarm horn, hour meter, 2 booster pumps, feedwater valve and strainer kits, back pressure regulators, chemical pump and tank (50 gallon), water softener, blowdown tank, blowdown cooling valve, chemical; skid mounted integrated unit needing only utility hookup to become operational

One Twin Unit CIF Bamako, Mali.....\$60,000.00

Two CLAYTON EO-33-1 UNITS ON SKID or equal

2. SPARE PARTS FOR ITEM 1.: to include those deemed to be required for uninterrupted operation but to include at least the following:

- a. chemical compound
- b. hoses
- c. fuses

Five Year Supply CIF Bamako, Mali.....\$1200.00

D. AUTOCLAVES

1. STERILIZER; gravity, single door for recessing; 24 x 36 x 60 inch size; steam; manual door operation; stainless steel panels; atmospheric exhaust; digital temperature (in Celsius) and chamber pressure (in metric) readout; meeting UL, CSA, ASME codes; compatible with item C,1.; controls to operate on 220V/1Ph/50Hz.

Two Each, CIF Bamako, Mali.....\$125,000.00

AMSCO EAGLE 3051 or equal

2. SPARE PARTS FOR ITEM 1.: to include those deemed to be required for uninterrupted operation but to include at least the following:

- a. spare circuit boards
- b. fuses
- c. lamps
- d. gaskets

Five Year Supply, CIF Bamako, Mali.....\$5000.00

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E. CLASSIC STILL

1. CLASSIC STILL; 38 lph capacity; electric powered; manual control with manual drain; with floor stand; 3/8 inch NPT cold water inlet; 1½ inch drain; for 380V/3Ph/50Hz operation, control voltage to be 220V/1Ph/50Hz.

One Each, CIF Bamako, Mali.....\$13,000.00

AMSCO MODEL 10 gph or equal

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F. CVL SPARE PARTS LIST

SPARE PARTS FOR MODEL 41 SUBLIMATOR

Address: The Virtis Company
Gardiner, NY 12525
914-255-5000

1. EXPANSION VALVES, CRE-4ZP, Sporlan

Four each, CIF, Bamako, Mali.....\$1,100.00

2. SOLENOID VALVES, Sporlan, #B6S1

Four each, CIF, Bamako, Mali.....\$482.00

3. FILTER DRYER, KMP083

Four each, CIF, Bamako, Mali.....\$133.00

4. OIL PUMP, 1420-0040

Two each, CIF, Bamako, Mali.....\$688.00

5. OIL SEPARATOR, 1140-0510

Two each, CIF, Bamako, Mali.....\$425.00

6. LIQUID INDICATORS, 1140-0410

Two each, CIF, Bamako, Mali.....\$48.00

7. SHELF TEMPERATURE CONTROLLER, 1510-0805

One each, CIF, Bamako, Mali.....\$460.00

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SPARE PARTS FOR MODEL 101 SUBLIMATOR

Address: The Virtis Company
Gardiner, NY 12525
914-255-5000

1. SUCTION ACCUMULATOR, 1140-0700-00

One each, CIF, Bamako, Mali.....\$143.00

2. INTERNAL COOLER, 10 HP, Copeland, 380V/3 Ph/50 Hz

One each, CIF, Bamako, Mali.....\$1,255.00

3. CONDENSER, Water Cooled, 1120-0720-00

One each, CIF, Bamako, Mali.....\$1,150.00

4. SOLENOID VALVE, 1150-0915-00

Four each, CIF, Bamako, Mali.....\$480.00

5. HEATING FLUID, DC200, 3810-0005-00

10 Gallons, CIF, Bamako, Mali.....\$1,260.00

6. REFRIGERATION DRYER, 1140-0140-00

Four each, CIF, Bamako, Mali.....\$133.00

7. EXPANSION VALVE, 1150-0114-00

One each, CIF, Bamako, Mali.....\$400.00

8. OIL SEPARATOR, 1140-0510-00

One each, CIF, Bamako, Mali.....\$212.00

9. DANFOSS OIL SEPARATOR, 1140-0500-00

One each, CIF, Bamako, Mali.....\$170.00

10. LIQUID EYE, 1140-0400-00

One each, CIF, Bamako, Mali.....\$25.00

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REFRIGERANTS FOR SUBLIMATORS

Address: Harry Alter, Inc.
49-01 Masbeth Avenue
Masbeth, Queens, NY 11378

1. FREON 13B1, 28 lbs

One each, CIF, Bamako, Mali.....\$315.00

2. FREON 13B1, 150 lbs

One each, CIF, Bamako, Mali.....\$1,500.00

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SPARE PARTS FOR INGERSOLL-RAND COMPRESSOR TYPE 30
MODEL 235 HNL

Address: Ingersoll-Rand Company International Export
91 New England Avenue
Piscataway, NJ 08854

1. SEAL, X1220T13

Four Each, CIF Bamako, Mali.....\$30.00

2. UNLOADER, 3A600C

Two Each, CIF Bamako, Mali.....\$500.00

3. CAP, 3A612

Two Each, CIF Bamako, Mali.....\$450.00

4. WEIGHT, 2A608

Four Each, CIF Bamako, Mali.....\$100.00

5. VALVE, 2A611

Four Each, CIF Bamako, Mali.....\$80.00

6. VALVE, 3W6874

Eight Each, CIF Bamako, Mali.....\$24.00

7. SWITCH, 9013GHG2

Two Each, CIF Bamako, Mali.....\$100.00

8. V-BELT, 3A19A71

Six Each, CIF Bamako, Mali.....\$90.00

9. CHECK VALVE, 3W11254P2T1

Two Each, CIF Bamako, Mali.....\$3.00

10. ELEMENT, 2A252

Four Each, CIF Bamako, Mali.....\$4.00

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SPARE PARTS FOR VACUUM PUMP

Address: Central Scientific Co.
International Division
2600 S. Kostner
Chicago, IL 60623
312-277-8300

1. VACUUM PUMP, HYVAC 45,115/230V,50Hz

One Each, CIF Bamako, Mali.....\$1500.00

2. LIGHT PUMP OIL, 1 Gallon

Ten Gallons, CIF Bamako, Mali.....\$75.00

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SPARE PARTS FOR AMSCO STERILIZER TYPE MF366, ORD
SERIAL # 288 663, DOUBLE DOOR

Address: American Sterilizer Company
2424 W. 23rd Street
Erie, PA 16514
817-452-3100
800-333-8838

1. VALVE, HI-LO, 96360-091

Two Each, CIF Bamako, Mali.....\$N/A

2. TUBE, BEND-TO-SUIT, 91808-091

Two Each, CIF Bamako, Mali.....\$N/A

3. NUT UNION, 2903-091

Two Each, CIF Bamako, Mali.....\$N/A

19

#####

G. VIRICIDAL TEST PROCEDURE: Excerpt from U. S. Code of Federal Regulations Part 9CFR:113.35. Detection of Viricidal Activity

(b) The product shall be tested with each virus fraction for which it is to be used as a diluent. If the vaccine to be rehydrated contains more than one virus fraction, the test shall be conducted with each fraction after neutralization of the other fraction(s) and/or dilution of the vaccine beyond the titer range of the other fraction(s). or the test shall be conducted using representative single fraction desiccated vaccines which are prepared by the licensee...

of the vaccine with the liquid product under test according to label recommendations and pool the contents. (2) Rehydrate at least two vials of the vaccine with the same volume of sterile purified water and pool the contents. (3) Neutralize to remove other fractions, if necessary. (4) Hold the two pools of vaccine at room temperature (20 to 25 C) for 2 hours. The holding period shall begin when rehydration is completed. (5) Titrate the virus(es) in each pool of vaccine as provided in the (protocol of production). (6) Compare respective titers.

(d) If the titer of the vaccine virus(es) rehydrated with the product under test is more than $0.7 \log_{10}$ below the titer of the vaccine virus(es) rehydrated with sterile purified water, the product is unsatisfactory for use as diluent.

(e) If the product is unsatisfactory in the first test, one retest to rule out faulty technique may be conducted using four vials of the vaccine for each pool and the acceptability of the product judged by the results of the second test...

#####

H. VACUUM TESTER

1. VACUUM LEAK DETECTOR: Continuous Duty, with separately housed transformer to isolate line voltage from output, 120 V/50 cycle operation (will require stepdown transformer for use at CVL but allows continuous operation)

Two Each, CIF Bamako, Mali.....\$1000.00

THOMAS SCIENTIFIC 9675-M10 or equal

80'

or, alternatively

1. VACUUM LEAK DETECTOR; Intermittent Duty (4 minutes "on", 5 minutes "off"); 208/220V/50 cycle

Six Each, CIF Bamako, Mali.....\$800.00

THOMAS SCIENTIFIC 9675-L13 or equal

#####

I. MOISTURE TESTING EQUIPMENT

1. DRY BOX: with interchange box, glove ports, and purge valves; 127Wx76Dx94cmH.

One Each, CIF Bamako, Mali.....\$7000.00

LABCONCO 50004 or equal

or, alternately

1. DRYBOX; with interchange box and purge valves; as above but fabricated locally from plexiglas with gasketed seals on interchange doors and window locks to maintain low humidity integrity

One Each, CIF Bamako, Mali.....\$N/A

2. RUBBER SLEEVES AND GLOVES: 20 cm; for item 1; accordion; size 9; with pack of 12 replacement latex gloves

Two Pairs, CIF Bamako, Mali.....\$450.00

LABCONCO 50059 or equal

3. HEATLESS FRACTIONATOR; based upon 32 ft³ dry box volume, 5.8 cfm air flow, nominal 30 minute dry-down complete with built-in air compressor and tank, with 2 sets of replacement columns.

One Each, CIF Bamako, Mali.....\$7500.00

MODEL HF315-A-120 or equal

PURE GAS EQUIPMENT CORPORATION

General Cable Apparatus Division

Post Office Box 666

5600 West 88th Ave.

Westminster, CO (80030)

303-427-3700

4. BALANCE: 0 to 200 g; +/- 0.03 mg precision; dimensions capable of being placed within item 1

One Each, CIF Bamako, Mali.....\$2000.00

METTLER AE50 or equal

5. WEIGHING BOTTLES: 35 ml capacity; case of 6, with stoppers

Six Cases, CIF Bamako, Mali.....\$650.00

KIMBLE 15165 50/12 or equal

6. TONGS: General Purpose

Three Each, CIF Bamako, Mali.....\$24.00

FISHER 15-202 or equal

7. SPATULA: 15 cm

Three Each, CIF Bamako, Mali.....\$12.00

FISHER 14-375-55 or equal

8. VACUUM OVEN: temperatures to 200 C; 0.45 ft³ capacity; capable of vacuum range down to 10 microns; 240V/50 Hz, with 2 spare door gaskets and one spare thermometer

One Each, CIF Bamako, Mali.....\$1400.00

NAPCO MODEL 5831 or equal

9. VACUUM PUMP: direct drive pump; 20 l/min free air displacement; 220v/50 Hz

One Each, CIF Bamako, Mali.....\$750.00

PRECISION MODEL DD-20 or equal

10. TUBING: clear; vacuum; 9.5 mm (3/8") ID; 3 m pack

Two Packs, CIF Bamako, Mali.....\$250.00

FISHER 14-169-2C or equal

11. DESICCANT: Drierite; indicating; 8 mesh; 5 lb bottle

One Bottle, CIF Bamako, Mali.....\$15.00

FISHER 07-578-3B or equal

42'

12. DRYING TUBE: polyethylene; 203 mm; pack of 12
One Pack, CIF Bamako, Mali.....\$60.00
NALGE 6201-0800 or equal

13. MANUAL CRIMPER: for removal of aluminum seals; to fit
seals for 20mm O.D. serum bottles
One Each, CIF Bamako, Mali.....\$150.00
WHEATON 224300 or equal
#####

J. MOISTURE TEST PROTOCOL: Excerpt from 9CFR113.29 on page
40A following:

§ 113.29 Determination of moisture content in desiccated biological products.

Methods provided in this section shall be used when a determination of moisture content in desiccated biological products is prescribed in an applicable Standard Requirement or in the filed Outline of Production for the product.

(a) Final container samples of completed product shall be tested. Individual samples having a low net weight shall be pooled at the time of testing in order to attain a minimum of 100 milligrams of dried product.

(b) The weight loss of the sample(s) due to drying to a constant weight in a vacuum oven shall be determined. The recommended equipment is as follows:

(1) Numbered, low-form, flat-bottom weighing dishes with tight-fitting lids.

(2) Vacuum oven equipped with a vacuum pump, accurate thermometer, vacuum gage, and thermostat. A suitable drying device shall be attached to the inlet valve of the oven. The vacuum system shall have a manometer and flowmeter for proper regulation of flow and pressure.

(3) Balance, accurate to 0.1 mg (rated precision ± 0.01 mg).

(4) Dry box or hood equipped with a suitable drying device and hygrometer to assure a relative humidity of 0 to 10 percent. The box should be sufficient in size to allow for the convenient transfer of samples and, if possible, incorporation of the balance and vacuum oven.

(5) If needed, a desiccator jar equipped with P_2O_5 plus $CaSO_4$ or $CaCl_2$, drying agent with indicator.

(c) Test procedure:

(1) Thoroughly cleaned weighing dishes shall be dried approximately 3 hours at 60° C under vacuum or for 1 hour at 100° C or higher in a drying oven. Immediately upon removal, the dishes shall be placed in a dry atmosphere and allowed to cool to room temperature. The tare weight of each weighing dish shall be determined as rapidly as possible. All manipulations of weighing dishes shall be made with tongs or while wearing gloves.

(2) The relative humidity of the dry box shall be reduced to 0 to 10 percent to assure a low level of moisture in the box during the time of transfer of the sample to the weighing dish.

(3) After the sample container has equilibrated in the dry atmosphere, the vacuum shall be released slowly allowing dry air to enter the bottle.

(4) The stopper shall be removed and the sample plug broken up with a spatula.

(5) The sample shall be rapidly transferred to a previously weighed and marked weighing dish and covered with its lid.

(6) After transfer has been completed, the weighing dish with its contents shall be weighed immediately giving the gross weight of the dish and sample. This weight minus the tare weight of the weighing dish is the sample weight.

(7) The lids shall be removed and placed with their matched weighing dishes in the vacuum oven. The pressure in the oven shall be reduced to 1 millimeter of mercury or less and the thermostat set at 60° C. A small amount of dry air shall be allowed to bleed into the oven during the drying period and the reduced pressure maintained by continuous operation of the vacuum pump.

(8) After 24 hours of drying time, the vacuum pump shall be stopped and dry air allowed to continually bleed into the oven with an increased flow rate until the pressure inside of the oven has been equalized with the atmosphere.

(9) The lids shall be immediately replaced in the normal closed position and the dishes placed in an efficient desiccator and allowed to cool to room temperature.

(10) Immediately upon reaching ambient temperature, each weighing dish containing the sample shall be removed from the desiccator and weighed as rapidly as possible. This weight subtracted from the gross weight obtained in accordance with paragraph (c)(6) of this section gives the equivalent weight of moisture lost upon drying.

(11) The steps prescribed in paragraphs (c)(8), (9), and (10) of this section may be repeated if experience has indicated that the particular product will not dry to a constant weight in the first 24 hours.

(12) Refer to paragraphs (c)(6) and (10) of this section. The equivalent weight of moisture divided by the sample weight times 100 equals the percentage of moisture in the original sample.

#####

K. NAMES AND ADDRESSES OF POSSIBLE TRAINING SITES

1. Mr. Thomas Freeze, President
Diamond Scientific Company
Post Office Box 328
2538 SE 43rd Street
Des Moines, IA 50302
515-262-9341
- 2 Dr. Daniel Gaudry
Rhone-Merieux, Inc.
117 Rowe Road
Athens, GA 30601
404-549-4503
3. Dr. Marty Vanier, Science Coordinator
Animal Health Institute
119 Oronoco Street
Box 1417-D50
Alexandria, VA 22313
703-684-0011
4. Mr. Darryl Peterson
The Virtis Company, Inc.
Gardiner, NY 12525
800-431-8232
914-255-5000
5. Export Accounts/Managing Office
American Sterilizer Company
2425 West 23rd Street
Erie, PA 16512
800-333-8838
814-452-3100
6. Clayton Manufacturing Company
P. O. Box 550
El Monte, CA 91734
415-782-0283
7. Everett "Ray" Taggart
5171 SE 40th Street
Allen Township
Des Moines, IA 50317
515-285-5249

33

8. Dr. Charles Mebus
Plum Island Animal Disease Center, North Atlantic Area
P. O. Box 848
Greenport, L.I., NY 11944-0848
516-323-2500

#####

L. SPECIFICATIONS FOR PLASTIC BOTTLES

Specifications are on Pages 42A1-42C3 following:

46'



PHILLIPS CHEMICAL COMPANY
A DIVISION OF PHILLIPS PETROLEUM COMPANY
PLASTICS TECHNICAL CENTER
Bartlesville, Oklahoma 74004

TECHNICAL
INFORMATION
ON
MARLEX
RESINS

MARLEX HHM 6502 . . . HIGH DENSITY ETHYLENE HEXENE-1 COPOLYMER

42 A

CUSTOMER BENEFITS

This resin allows the blow molder to reduce inventory of resin types, because it can be used to package bleach and most detergents.

Compare this with other blow molding or thermoforming resins of the same stiffness. Note the . . .

- * Excellent stiffness
- * Exceptional stress cracking resistance

SUGGESTED APPLICATIONS

Bottles for . . .

- * Bleach and detergents
- * Industrial chemicals
- * Industrial parts

PROCESSING RECOMMENDATIONS

Maintain these conditions for optimum part quality.

Blow Molding stock temperature

* 340-400°F/171-204°C

Extrusion melt temperature

* 380-420°F/194-216°C

Thermoforming surface temperature

* 340-360°F/171-182°C

SPECIFICATION DATA

Meets these requirements .

- * ASTM D1248 - Type III, Class A, Category 5
- * FDA Regulation 177.1520, Suitable for food packaging.

|||||

(9)

NOMINAL PHYSICAL PROPERTIES OF MARLEX HHM 5502

12 ft.

| PROPERTY* | ASTM | English Units | English Value | Metric Units | Metric Value |
|---|-----------------------|---------------------|---------------|-------------------|--------------|
| Density | D1505 | lbs/ft ³ | 59.6 | g/cm ³ | 0.955 |
| Melt Index | D1238 | g/10 min. | 0.35 | g/10 min. | 0.35 |
| Flow Index (CIL, 375°F/190°C 1500 psi/10.4 MPa) | (1) | g/10 min. | 2.. | g/10 min. | 3.0 |
| ESCR, Condition A, F50 | D1693 | hr | 45 | hr | 48 |
| Tensile Strength @ Yield 2" (50.8 mm) per min. | D638 Type IV Spec. | psi | 4000 | MPa | 88 |
| Elongation 2" (50.8 mm) per min. | D638 Type IV Spec. | % | > 600 | % | > 800 |
| Brittleness Temperature | D746 | °F | <-180 | °C | <-118 |
| Flexural Modulus | D790 | psi | 200,000 | MPa | 1,880 |
| <u>Bottles</u> | | | | " | " |
| Bottle Environmental Stress Cracking Resistance (2), 140°F/60°C, F50 | | hr | 250 | hr | 250 |

Thermoforming

Sheet Sag

2" x 4" x 125 mils (81 mm x 122 mm x 3.8 mm)
thick blank heated to forming temperature inches 7-9 cm 18-23

* Physical properties reported herein were determined on compression molded specimens prepared in accordance with Procedure C of ASTM D1928.

(1) Data obtained using a gas operated extrusion plastometer based on a design by Canadian Industries, Ltd., with a die having an orifice diameter of 0.01925"/0.49 mm and a land length of 0.178"/4.48 mm.

(2) Test Conditions: 10-ounces, 23-gram bottle, 10% fill, Orvus X detergent.

This document reports accurate and reliable information to the best of our knowledge, but our suggestions and recommendations cannot be guaranteed because the conditions of use are beyond our control. Information presented herein is given without reference to any patent questions which may be encountered in the use thereof. Such questions should be investigated by those using this information. Phillips Petroleum Company assumes no responsibility for the use of information presented herein and hereby disclaims all liability in regard to such use.

MAY, 1970

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MECHANICAL SPECIFICATION

412B

CODE NO. 07-817-09

ISSUED 10-7-86

COMPONENT P/E Serum

REVISION NO. Additional

SIZE 30 ml - 20 ml fill.

SUPERSEDES NA

PRODUCT NAME Various

APPROVED BY Robert Z. Bush

MECHANICAL SPECIFICATIONS:

Style - Serum

Material - High Density Polyethylene

Resin - Marlex 5502

Pigment - Natural

Treatment - Flamed

Finish - 20 mm serum

Capacity - 30 ml

Weight - 6.0 gm \pm .5 gm

Height - 2.615 \pm .047"

O.D. - 1.235" \pm .031"

Neck - I.D. .492" to .508"

Drawing Number - 21574

PRINTING SPECIFICATIONS:

NONE

PACKAGING SPECIFICATIONS:

Bulk pack in P/E bags, overpacked in corrugated cases of uniform count.

Mark with Diamond P. O. #

Item Description

Quantity

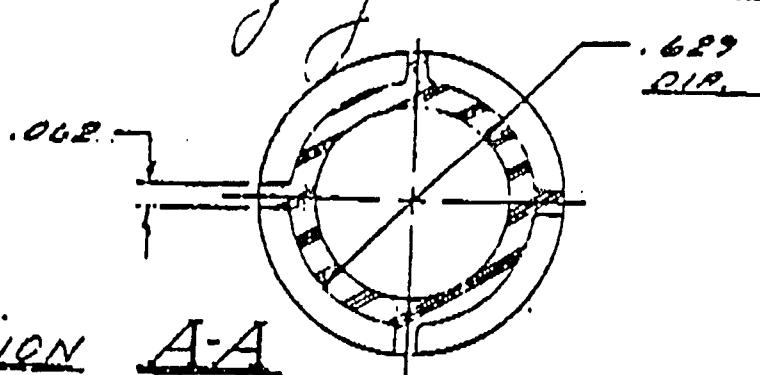
Code No. 07-817-09

Recommended Vendor: Wheaton Plastic

89'

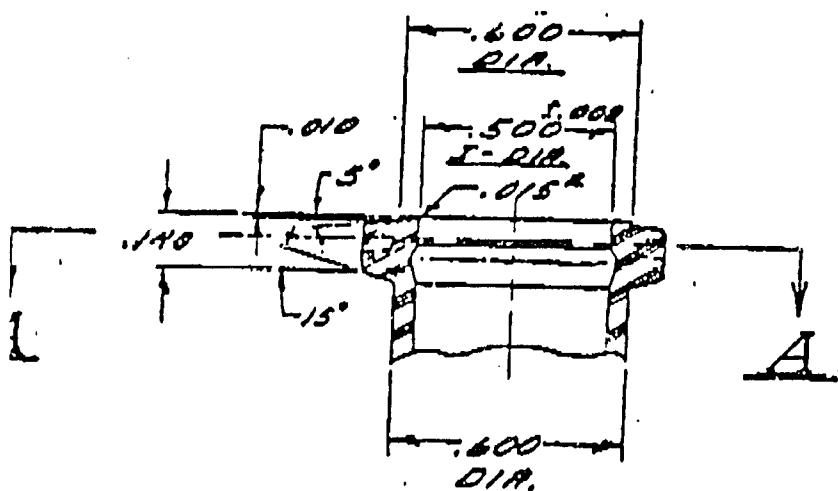
O Brdg.

PROJ. N° 76-8



NOTE: 4262
TOLERANCES APPLY TO THE AVE
HIGH & LOW POINTS ON DIAMETER.

| | C.R.P. |
|-----------------|------------|
| FLAMED ONLY | 37 ° 1 : 1 |
| FLAMED & LEHRED | 36 ° 1 : 1 |



Northwestern Bottle Co.
9850 Industrial Blvd.
Leavenworth, Kansas 66215

NECK DETAIL
SCALE: 2:1

REGULAR SEMI 2210-208 NECK FINISH
CAPACITY: SEE CHART
WEIGHT: 6.45 POUNDS

100 BOTTLE

SCALE FULL
LINE (HIGH DENSITY)

TOLERANCES
UNLESS SPECIFIED

1215 TRAP +

DRW. BY C.L.M.

DATE 2-10-77

CHECKED BY

JY '66 RODEO
PRELIMINARY
NOTE REMOVED

REV.
LET.

REVISION DESCRIPTION

F.C.

B.S.

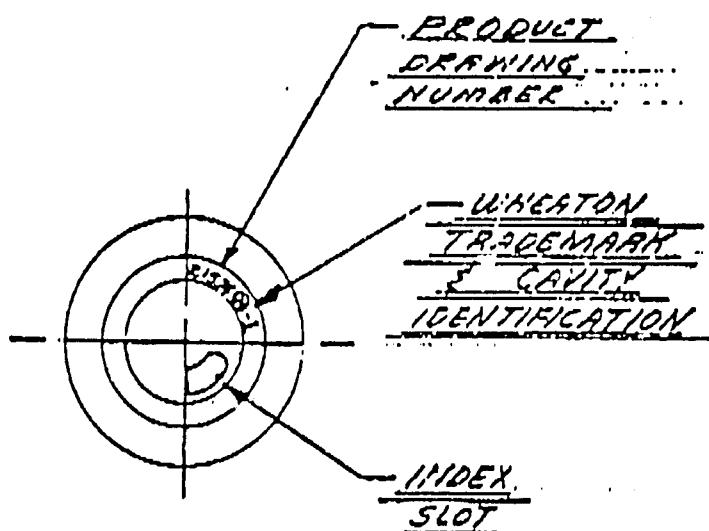
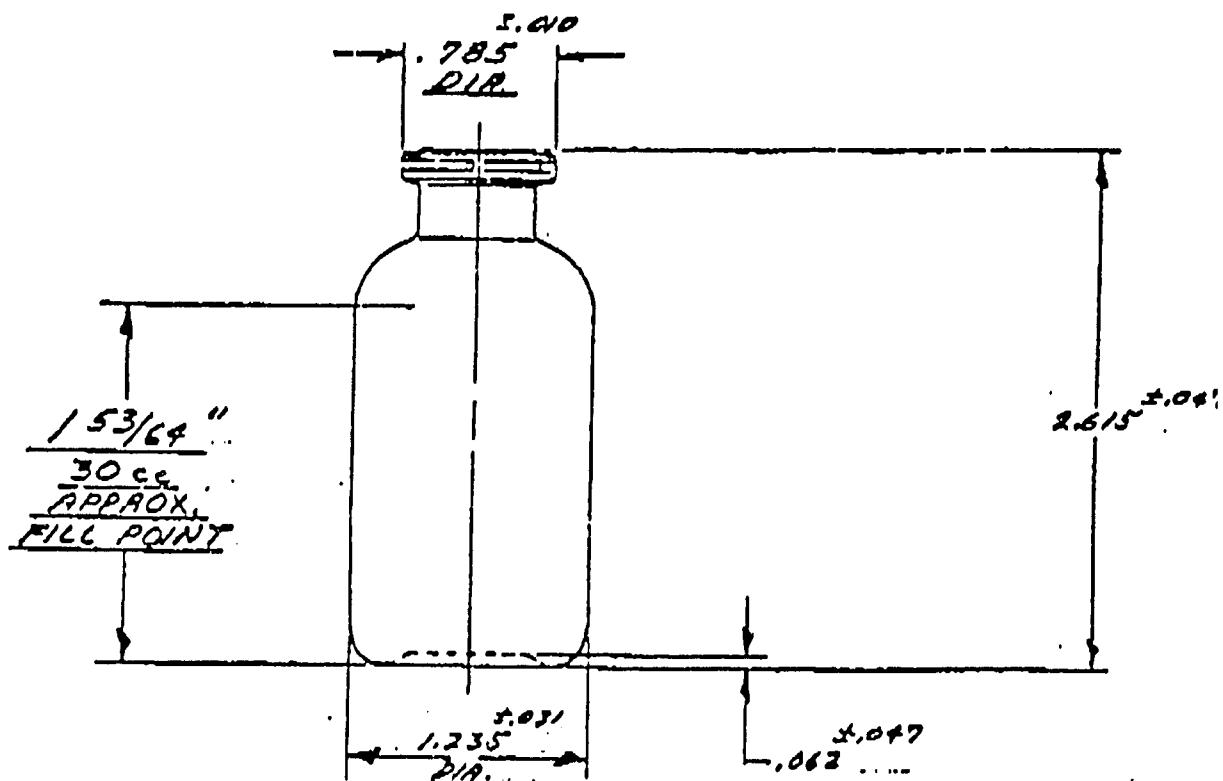
BY

WHEATON PLASTICS CO.
MAYS LANDING, N.J.

DWG - - -

1
.00

42B3



| | |
|----------|--------------|
| TITLE | 30 cc SER. |
| CUSTOMER | STOCK |
| MATERIAL | POLYETHYLENE |

42C1

MECHANICAL SPECIFICATION

CODE NO. 07-824-15

ISSUED 10-7-86

COMPONENT Polyethylene Serum Bottle

REVISION NO. Additional

SIZE 120 ml - (round flat)

SUPERSEDES NA

PRODUCT NAME Various

APPROVED BY Dale J. Ziegler

MECHANICAL SPECIFICATIONS:

Style - Polyethylene Serum bottle

Materials - High Density Polyethylene

Resin - Marlex 5502

Pigment - Natural

Treatment - Flamed

Finish - 20 mm Serum

Capacity - 120 ml

Weight - 13 gm ± .5 gm

Height - 4.05" ± .047"

Diameter - 1.850" ± .031"

Neck - I.D. .492" to .508"

Drawing Number - 21539

PRINTING SPECIFICATIONS:

NONE

PACKAGING SPECIFICATIONS:

Bulk packed in corrugated cartons of uniform count lined with poly bag.

Mark with Diamond P. O.

Item Description

Quantity

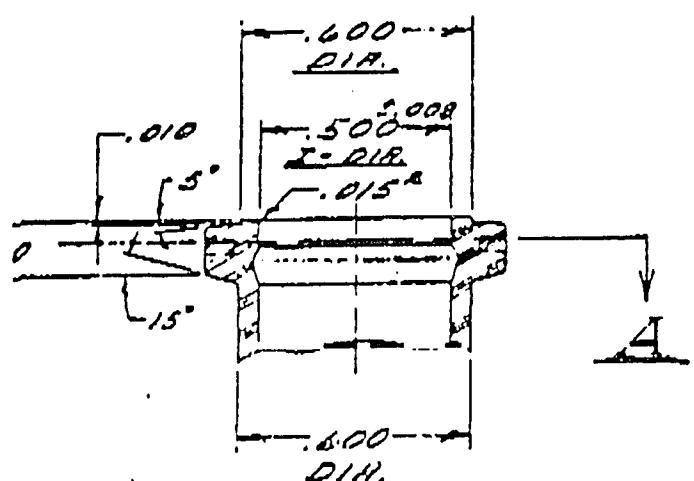
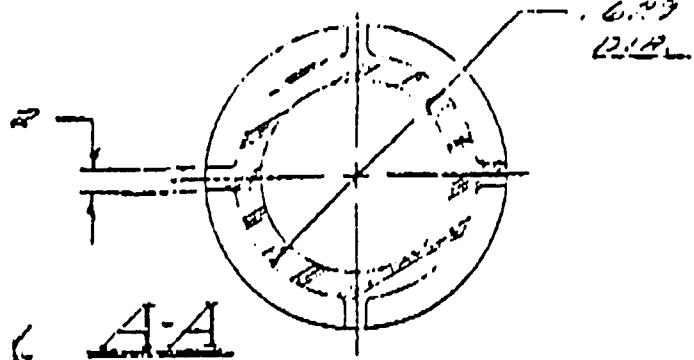
Code No. 07-824-15

Recommended vendor: Wheaton Plastic

42'

Schaefer Kite Planes PROJ. 117 PG. 04

4LC 2



NOTE:

TOLERANCES APPLY TO THE AVERAGE OF
HIGH & LOW POINTS ON DIAMETER(S)

| | FLANGE DIA. | SP. O.D. |
|---|--------------------------|----------|
| ① | FLANGE DIA. | .185 .00 |
| ② | FLANGE DIA. LENE.R.ED | .182 .00 |

Northwestern Bottle Co.
9850 Industrial Blvd.
Leavenworth, Kansas 66210

NECK DETAIL
SCALE: 2:1

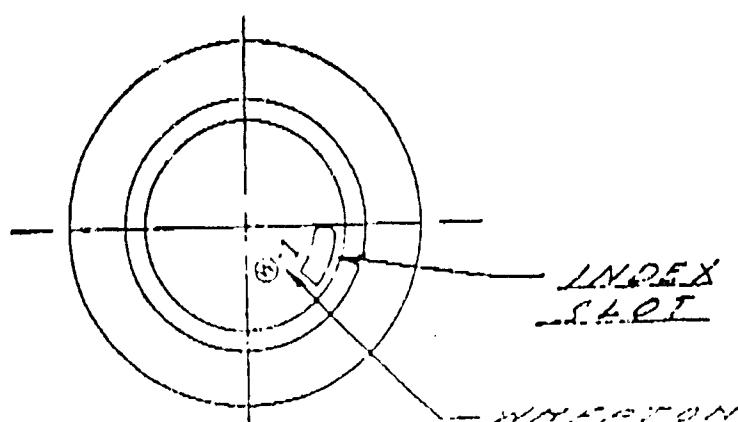
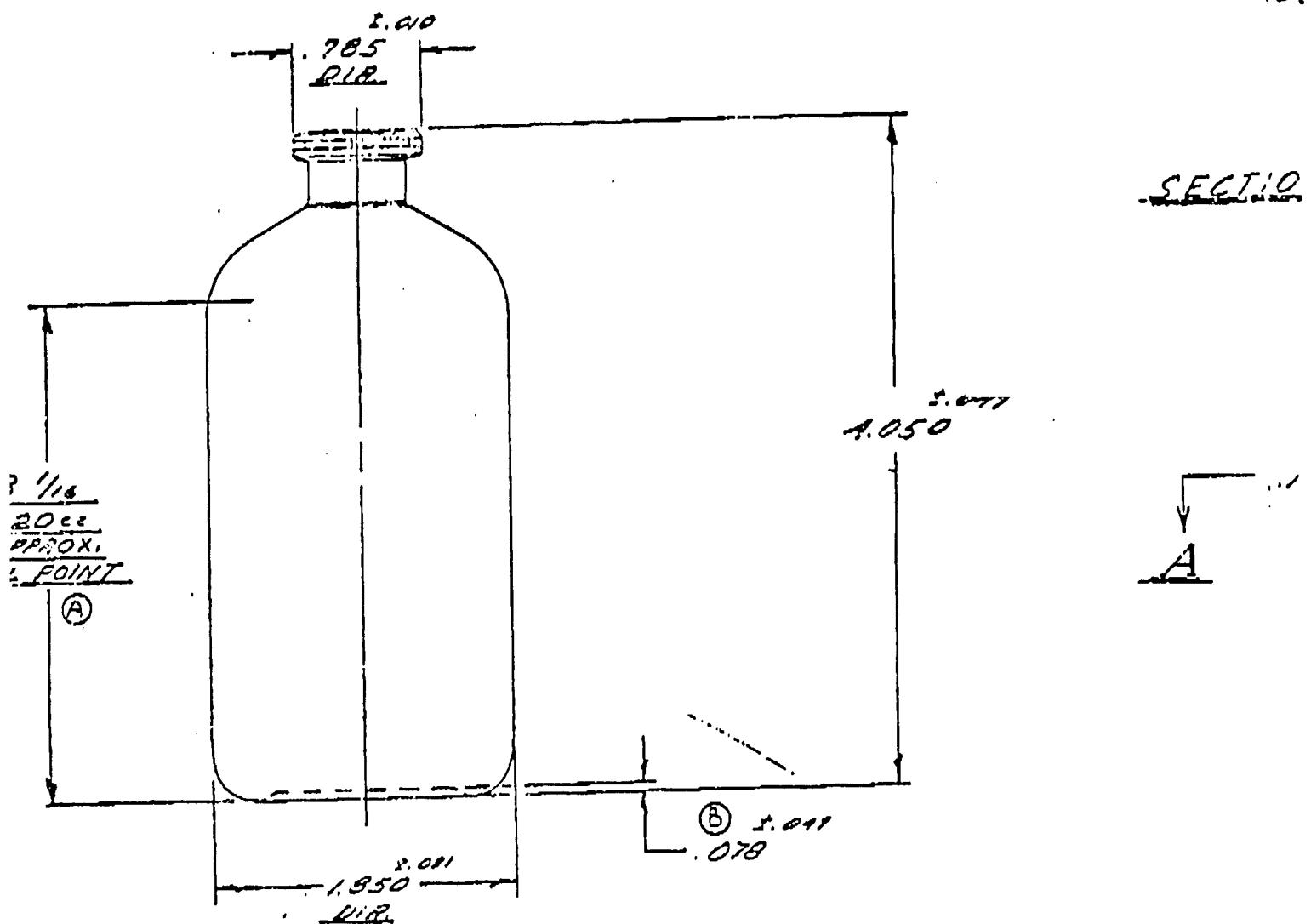
11-6101-3710-202 NECK FINISH
WTG: SEE CHART
ST. 15.0 51.975

| | | | |
|-----------------------------------|--|-----------|---|
| PRINTED | DRW. BY M.C.J. | ④ | RECOMMENDED NOTE REMOVED FILL POINT ADDED |
| SCALE, ETC. | DATE 11-25-74 | | |
| 116.11 115.11 117.11 | CHECKED BY M.C.J. | REV. LST. | REVISION DESCRIPTION |
| NO. 28 SPECIFIED IAC. # 117 | WHEATON PLASTICS CO. MAYB LANDING, N.J. | | DWG. B-21559 |

93

42 C3

.06



EXTRACTED
24 INCH DRAWING
CHECKED
ACCURATELY

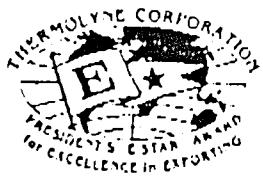
SP-500
G.C.P.
③ P.W.E.

| | |
|----------|-----------------------------|
| TITLE | 42 C3 |
| CUSTOMER | S T C S |
| MATERIAL | F-O-L Y F I N E R. 400 |
| | TOLE UNLIC DIE R. 100 |

11220 A

64

Barnstead | Thermolyne



412

DATE 6/9/89

NAME: JAMES A. GOURLAY, DVM

COMPANY: PHONE 415-523-3911

SENDERS NAME: KIM MURDOCK-TIMMERMAN
BARNSTEAD/THERMOLYNE
FAX NO. 319 556 0695

NUMBER OF PAGES INCLUDING COVER SHEET: 1

COMMENTS:

RE: WATER ANALYSIS RESULTS FOR BAMAKO, MALI, W.AFRICA
AS REQUESTED BY JAMES A. GOURLAY, DVM

RESISTIVITY, R25.....6,578 ohm/cm

T.D.S.....85.88 ppm

pH.....7.9

T.O.C. (NPOC + POC).....2.98 ppm (as C)

CO2.....1.02 ppm

CALCIUM.....17.50 ppm

ALKALINITY.....35.00 ppm

SAMPLE NUMBER 205331....TESTED 6/8/89

JIM...YOUR HARD COPY AND RECOMMENDATION WILL FOLLOW BY MAIL. PLEASE PHONE
IF YOU NEED ADDITIONAL ASSISTANCE.

A handwritten signature that appears to read "Kim" or "Kimm". It is written in cursive ink above a horizontal line.

Postal Address: P.O. Box 707, Dubuque, Iowa 52004-0797 USA
Street Address: 2555 Körper Blvd., Dubuque, Iowa 52001-1451 USA
TELEPHONE (319) 558-2241 TELEFAX (319) 558-0695
Cable Address: THERMOLYNE Dubuque, Iowa, TELEX 284767 THERMUR

WB

Thermostability of A Vero Cell-Adapted Rinderpest
Vaccine with Optimized Chemical Stabilization

Jeffrey C. Mariner, James A. House*, Albert E. Sollod·
Chip Stem, Marinus van den Ende and Charles A. Mebus**

Tufts University School of Veterinary Medicine, Section of
International Veterinary Medicine, North Grafton,
Massachusetts, 01536 , *Animal and Plant Health Inspection Service
and **Agricultural Research Service, U.S.D.A. both located at
Plum Island Animal Disease Center, Box 848, Greenport, New York,
11944

Corresponding Author: Dr. Jeffrey Mariner

Plum Island Animal Disease Center
Box 848
Greenport, NY 11944
(516) 323-2500 x314

4/6

Abstract:

Mariner, Jeffrey C., House, James A., Sollod, Albert G., Stem, Chip, Van den Ende, Marinus, and Mebus, Charles A. 1988. Thermostability of a Vero Cell-Adapted Rinderpest Vaccine with Optimized Chemical Stabilization

The thermostability of a rinderpest vaccine produced on Vero cells was evaluated using a variety of chemical stabilizers and lyophilization protocols. Three stabilizer preparations and two lyophilization schedules were examined using accelerated stability testing at 37C. The stabilization method and high Vero cell virus batch titers resulted in a lyophilized vaccine which maintained the minimum required dose of log₁₀ 2.5 TCID₅₀ for longer than eight weeks at 37C.

Introduction:

Rinderpest (RP) has remained a major threat to animal agriculture in the developing world despite extensive efforts at control and eradication. Programs designed to control RP are often the single largest undertaking of the national veterinary services in less developed countries. The Plowright vaccine has been the most efficacious tool at the disposal of these programs. This vaccine is produced from the Kabete O strain of RP virus attenuated by 92 passages in bovine kidney cells (Plowright and

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Ferris, 1962). It causes no clinical sign in cattle, is not transmitted by natural means and a single vaccination results in lifelong immunity (Plowright, 1984). The only apparent defect of the Plowright vaccine is its lack of thermostability, even in the lyophilized state, resulting in the requirement of continuous refrigeration (Anonymous, 1985a). This requirement, the 'cold chain', is one of the highest recurrent costs associated with RP vaccination campaigns. The proposal to make the cold chain obsolete by development of a thermostable vaccine was advanced by the Niger Integrated Livestock Production Project as an integral part of the project (Soliod et al., 1983). Elimination of the cold chain requirement from African RP campaigns has an estimated potential savings of 3.6 million dollars per year (Strayker et al., 1988).

Numerous studies on the thermostability of rinderpest vaccines, produced on bovine kidney cells and lyophilized with a variety of chemical stabilizers, have been performed. The most complete study was done by Plowright (1970), which included a summary of previous findings from the literature. Plowright found 5% lactalbumin hydrolysate and 10% sucrose to be a superior additive. Our preliminary work, done at Plum Island Animal Disease Center, found this stabilizer to be superior to other traditional additives. These included an N2 amine formulation and the sucrose, potassium phosphate buffer, L-glutamine and bovine

albumin stabilizer (SPGA).

In recent years a number of measles vaccines have been developed which have improved thermostability (McAleer et al., 1980; Colinet and Peetermans, 1982). One of these 'second generation measles vaccines' has been tested under field conditions in Cameroon and found to be immunogenic after two weeks at a controlled ambient temperature (23.1-25.4C) (Heymann et al., 1979). This improvement has been attributed to a buffered hydrolyzed gelatin and sorbitol stabilizer in at least one second generation vaccine (McAleer et al., 1980).

It was the purpose of this study to evaluate and report on the thermostability of Vero cell-adapted RP vaccine prepared with three different chemical stabilizers. They were: (1) lactobionic acid, hydrolyzed gelatin and sorbitol (LGS), (2) 5% lactalbumin hydrolysate and 10% sucrose (LS), (3) buffered hydrolyzed gelatin and sorbitol (BUGS). The lyophilization schedule was optimized and related to residual moisture for each of the vaccine preparations. The results are discussed in relation to previously reported thermostability values for RP vaccines produced on bovine kidney cells.

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Materials and Methods:**Vaccine:**

Rinderpest virus of the Plowright Kabete O strain was passed 4 times on Vero cells obtained from the National Veterinary Service Laboratory, Ames, Iowa. The fourth passage was made in 760 cm² roller bottles and harvested for vaccine production at 90% cytopathic effect (CPE) on the fifth day. Eagles minimum essential media supplemented with 10% fetal bovine serum was used as the growth medium.

Two batches of vaccine were produced and designated 5/88 and 6/88. Infected cell monolayers were removed with glass beads and the resulting cell and virus suspensions were pooled for each batch. Aliquots of the virus and cell suspension were combined with each stabilizer in bulk prior to freezing at -70C for storage until lyophilization. At the time of lyophilization at least two one ml samples were taken for titration. Each batch was lyophilized using different time and temperature parameters. Batch 5/88 was brought to a final temperature of 30C for a total cycle of 72 hours. Batch 6/88 was raised to 35C during a cycle of 74 hours duration. Residual moisture was determined for each preparation of each batch by combining and weighing the contents of five vials. This material was placed in a desiccator for at least two weeks and then weighed again.

The LS stabilizer consisted of 5% lactalbumin hydrolysate and 10% sucrose with the pH adjusted 7.2 using potassium hydroxide. The BUGS stabilizer contained 3.5% hydrolyzed gelatin and 3.5% D-sorbitol with 0.1M potassium phosphate buffer at pH 6.2 (Protected by US patent #4,147,772, Merck and Co., Inc., Rahway, New Jersey). The LGS additive was a complex mixture of equal parts of three solutions: (1) 20% lactose, (2) 20% hydrolyzed gelatin and (3) 12.5% lactobionic acid, 8.75% D-sorbitol and 1.19% HEPES buffer. The pH of the third component was adjusted to 7.2 using potassium hydroxide.

LS was combined with virus suspension in a 1:1 ratio and included in both batches (Plowright et al., 1969). LGS was used in a 1:1 ratio and only produced in the batch 5/88. Three formulations of vaccine were produced with the BUGS stabilizer. Two vaccines using a ratio of either 3 (3:1 BUGS) or 4 (4:1 BUGS) parts stabilizer to virus suspension were made in both batches 5/88 and 6/88. In batch 6/88 a more concentrated BUGS additive based on the final concentrations in the 4:1 vaccine from batch 5/88 was used in a 1:1 ratio (1:1 BUGS) to give the same final concentration of stabilizer components.

Accelerated Stability Test and Calculations:

Vaccine vials were placed in a 37C incubator (+/- 0.5C)

immediately following lyophilization. At least two vials were taken at each time point and stored at -20C until titrated. Samples were taken on days 4, 7, 14, 21, 28, 42 and 56 for both batches. An additional sample was taken on day 10 for batch 5/88 and day 35 for batch 6/88.

Degradation curves were analyzed using a two component model developed for measles vaccines (Allison et al., 1981). This method separates the degradation process into a rapid initial decay followed by a gradual linear component. The Statistical Analysis System (SAS) regression procedure was used to determine the degradation constant (k) and the y intercept of the linear component. The degradation constant is a measure of the second degradation component, thus titers from the 0 and 4 day time point were excluded from calculations of ' k '. SAS provided ratio of variance (F) and R-squared values allowing evaluation of the goodness of fit for each regression. A statistical comparison of different models using day zero, four and seven as the boundary between the two components was also preformed using analysis of variance. In this case, the ratio of variance (F) was determined using the mean squares for error provided by SAS for the different models. A t-test using the standard error values of the regression coefficients was performed to determine the significance of the difference between the degradation constants of different virus stabilizer preparations (Steel and Torrie, 1980). The initial loss was calculated as a measure of the first

degradation component. This was the difference between the y intercept for the regression line of the linear component and the actual titer of the unheated vaccine.

The corrected shelf-life was calculated as the difference between the y-intercept and the minimum permissible titer divided by the degradation constant. The minimum permissible titer per 50 dose vial was $4.2 \log_{10} \text{TCID}_{50} \text{ ml}^{-1}$. This was based on a minimum single dose of $2.5 \log_{10} \text{TCID}_{50} \text{ ml}^{-1}$.

Virus Titrations:

Virus titrations were carried out in microtiter plates using Vero cells. Two 10-fold dilution series were prepared for each sample using Eagles minimum essential media as a diluent. Six replicates were used per dilution with 25 μl of virus suspension being added to 100 μl of cell suspension ($1.5 * 10^5 \text{ cells ml}^{-1}$). All tests were incubated at 37°C in 5% CO₂ and read after seven days.

Results:

Figure 1 shows two plots of representative data illustrating the two component model. In the two cases illustrated, the model using day seven as the start of the linear component gave a significantly better fit than regression lines starting at day zero or four. In other cases, the R-squared values for the two

component model using day seven as the boundary were higher or comparable to those for models using day zero or four, but the ratios of variance (F) between these models were not generally significant. The results of the calculations of the degradation constant and the rapid initial loss are shown in Table 1. All degradation constants were statistically significant with the exception of the 6/88 LS preparation. This was due to the small size of the estimated 'k' value, thus, no significant decline in titer was detected between days 7 and 56 in this LS vaccine. The BUGS preparations as a group had smaller 1st component losses, however the k values for these vaccines were higher than for LS. A comparison of 1:1 BUGS and LS is shown in figure 2. The t-test found the degradation constants of LS to be significantly different from all other stabilizer preparations tested ($P < .05$). Based on the regression values the two LS preparations have corrected shelf-lives of 101 and 131 days at 37C.

Table 2 summarizes the losses during lyophilization and residual moisture for all vaccine preparations. Increasing the length and final temperature of the lyophilization cycle had a desirable effect on both components of the degradation curve without increasing the loss of virus infectivity. The differences in residual moisture measurements between different batches of the same stabilizer preparation correlated with the changes in stability parameters between batches for that stabilizer.

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Discussion:

The two component model for thermal inactivation has been described for several important viruses and has been reviewed by Woese (Woese, 1960). Of interest to our study is the report on two component analysis of second generation measles vaccine degradation curves (Allison et al., 1981; Colinet and Peetermans, 1982). This model was statistically shown to give a better fit than a completely linear model for two out of three second generation measles vaccines tested (Colinet and Peetermans, 1982). The model increases the accuracy of vaccine degradation comparisons and improves the estimates of vaccine shelf-life.

The k constant reported for BUGS stabilized measles virus at 3°C was reported as 0.0172 log₁₀ day⁻¹ (McAleer et al., 1980). This is roughly half the value we found for RP virus at the same temperature. McAleer does not state the residual moisture value for this vaccine so we cannot rule out a difference in efficiency of lyophilization. However, if one compares the values for 3:1 BUGS and 4:1 BUGS from our two batches, reduction in residual moisture did not have a large effect on degradation rates or shelf-lives.

The shelf-life estimates and 'k' values we obtained for LS

stabilized RP vaccine were better than any previously reported for either measles or RP vaccines. A study on the thermostability of a LS stabilized measles vaccine would be a worthwhile undertaking. The average of the five k values found by Plowright with LS stabilized RP vaccine at 37C was 0.0139 when converted to \log_{10} day⁻¹ (Plowright et al., 1970). If one reviews their graphical data presented for 37C a two component degradation is evident. Plowright did not take this into account in his calculations, however his measurements were made over a sufficiently long period of time (up to 36 weeks) to reduce any resulting error. Initial titers for the lyophilized vaccines produced on bovine kidney cells were lower with none described going far above 5.5 \log_{10} TCID₅₀. A corrected shelf-life at 37C, based on the presented regression equations, would be essentially zero days using current criteria for dose, thus the requirement of a continuous cold chain.

The extended life of the RP vaccines produced on Vero cells and stabilized with LS can be attributed to higher initial titers and optimized lyophilization resulting in good residual moisture values with minimal loss of titer during the process. In order to produce a vaccine with extended life at higher temperatures laboratories must examine their lyophilization cycles and routinely determine the level of efficiency in terms of dryness of the product and loss of titer during the cycle. Laboratories complying with FAO guidelines for quality control should already

have the titration information available (Anonymous, 1985b). The vaccine should be produced on Vero cells, which besides producing higher virus yields, has other advantages in terms of quality control and supply. Use of Vero cells is within current FAO guidelines (Anonymous, 1985c). Abbreviated stability tests should be conducted on each batch to assure sufficient shelf-life.

The reduced rapid initial losses for the 1:1 BUGS stabilizer results in a more gradual decline in titer during the early weeks of exposure. As shown in figure 2, the 1:1 BUGS stabilized vaccine maintained a higher titer than the LS stabilized vaccine up until day 33. This stabilizer would not require vaccine with as high an initial titer as the LS stabilized vaccine to achieve a more limited degree of thermostability. Vaccine production facilities finding it difficult to produce high titer virus may consider this an option.

Field conditions in Africa are noted for their high temperatures and often intense sunlight. Both of these have deleterious effects on RP vaccines. In Niger, for instance, the ambient temperature during the cool season when the RP vaccination campaign is operating often reaches 37C. Other times of the year temperatures climb as high as 45C.

The LS vaccine described here, if protected from the extremes of temperature and direct sunlight, should have a useful

life of one month at average ambient temperature and still maintain a wide margin of safety. A simple method of reducing temperature extremes is storage in burlap wrapped cases. The burlap is maintained moist and evaporative cooling reduces interior temperatures to an acceptable level without the use of ice. It is our intention to field test this vaccine under these conditions in the 1988 Nigerien RF campaign.

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Table 1

Comparison of Rinderpest Vaccine Degradation Values¹

| Batch | Stabilizer | n ² | k ^{3 4} | Y-Intercept ⁴ | Initial Loss ⁵ | Shelf-Life 37C ⁶ (days) |
|-------|------------|----------------|------------------|--------------------------|---------------------------|---------------------------------------|
| 5/88 | LS | 7 | -0.0116*** | 5.37 | 1.23 | 101 |
| | LGS | 7 | -0.0317*** | 5.00 | 1.54 | 25 |
| | 3:1 BUGS | 7 | -0.0381*** | 5.74 | 0.75 | 40 |
| | 4:1 BUGS | 7 | -0.0494*** | 5.89 | 0.54 | 34 |
| 6/88 | LS | 6 | -0.0078 | 5.61 | 1.18 | 181 |
| | 1:1 BUGS | 6 | -0.0361*** | 6.53 | 0.24 | 65 |
| | 3:1 BUGS | 6 | -0.0303*** | 5.59 | 0.65 | 46 |
| | 4:1 BUGS | 6 | -0.0363*** | 5.68 | 0.45 | 41 |

¹ All titers expressed as log₁₀ TCID₅₀ ml⁻¹² Sample size.³ k = the degradation constant (log₁₀ TCID₅₀ day⁻¹).⁴ Values were obtained from linear regression analysis on the degradation curves beginning at day 7.⁵ The difference between the initial titer and the y intercept of the regression line.⁶ Corrected shelf-life calculated from the regression line using 4.2 log₁₀ TCID₅₀ ml⁻¹ as the minimum titer per 50 dose vial and a minimum dose of 2.5 log₁₀ TCID₅₀.

*** P < 0.001.

Table 2

Summary of Vaccine Harvest Titers, Lyophilization Losses
and Residual Moisture for Rinderpest Vaccine¹

| Batch | Stabilizer | Harvest Titer ² | Lyophilized Titer | Loss ³ | Residual Moisture |
|-------|------------|----------------------------|-------------------|-------------------|-------------------|
| 5/88 | LS | 7.19 | 6.60 | 0.59 | 2.4% |
| | LGS | 7.02 | 6.54 | 0.48 | 1.3% |
| | 3:1 BUGS | 6.60 | 6.49 | 0.11 | 2.9% |
| | 4:1 BUGS | 6.69 | 6.43 | 0.26 | 3.5% |
| 6/88 | LS | 6.94 | 6.79 | 0.15 | 2.2% |
| | 1:1 BUGS | 6.85 | 6.77 | 0.08 | 2.7% |
| | 3:1 BUGS | 6.43 | 6.24 | 0.19 | 2.7% |
| | 4:1 BUGS | 6.41 | 6.13 | 0.28 | 3.0% |

¹ All titers expressed as log₁₀ TCID₅₀ ml⁻¹

² Bulk vaccine titer after combination with stabilizer and one freeze thaw cycle.

³ Loss in titer during lyophilization (harvest titer-lyophilized titer).

Figure 1. Degradation curves (37C) for lyophilized rinderpest vaccine stabilized with 3:1 buffered gelatin and sorbitol or 5% lactalbumin hydrolysate and 10% sucrose (Batch 5/88).

Figure 2. Comparison of the degradation values (37C) for rinderpest vaccine stabilized with 5% lactalbumin hydrolysate and 10% sucrose to rinderpest vaccine stabilized with 1:1 buffered gelatin and sorbitol (Batch 6/88). Plots represents the rapid initial loss (day 0 to 7) and the regression line of the linear component.

References

- Allison LMC, Mann GF, Perkins FT, Zuckerman AJ, 1981. An accelerated stability test procedure for measles vaccines. *J Biol Stand* 9:185-194.
- Anonymous, 1985a. Report of the FAO Expert Consultation on Rinderpest Diagnosis, Vaccine Production and Quality Control. Food and Agriculture Organization of the United Nations, Rome, 31-33.
- Anonymous, 1985b. Report of the FAO Expert Consultation on Rinderpest Diagnosis, Vaccine Production and Quality Control. Food and Agriculture Organization of the United Nations, Rome, 95-133.
- Anonymous, 1985c. Report of the FAO Expert Consultation on Rinderpest Diagnosis, Vaccine Production and Quality Control. Food and Agriculture Organization of the United Nations, Rome, 25.
- Colinet G, Peetermans J, 1982. Behavior of five commercial measles vaccines in an accelerated stability test. *J Biol Stand* 10:241-247.
- Heymann DL, Nakano JH, Maben GK, Durand B, 1979. Field trials

of a heat-stable measles vaccine in Cameroon. Br Med J ii:99-100.

McAleer WJ, Markus HZ, McLean AA, Buynak EB, Hilleman MR, 1980. Stability on storage at various temperatures of live measles, mumps and rubella virus vaccines in new stabilizers. J Biol Stand

Plowright W, 1984. The duration of immunity in cattle following inoculation of rinderpest cell culture vaccine. J Hyg Camb 92:285-296

Plowright W, and Ferris RD, 1962. Studies with rinderpest virus in tissue culture: the use of attenuated culture virus as a vaccine for cattle. Res vet Sci 3:172-182.

Plowright W, Herniman KAJ, Rampton CS, 1969. Studies on rinderpest culture vaccine II. Factors influencing the accuracy of vaccine·potency tests. Res vet Sci 10:502-508.

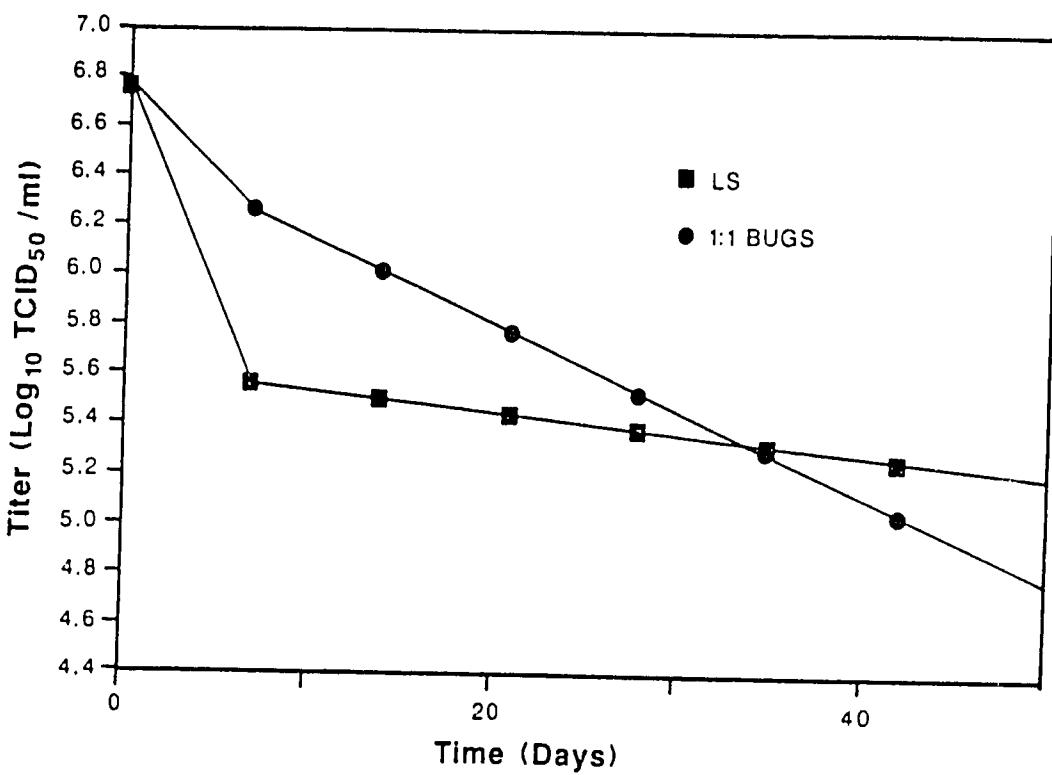
Plowright W, Rampton CS, Taylor WP, Herniman KAJ, 1970. Studies on rinderpest culture vaccine III. Stability of the lyophilized product. Res vet Sci 11:71-81.

Sollod A.E., De Haan C., and Stem E., 1983. Niger Integrated Livestock Production Project Paper. Annex B: Animal Production and Health, Tufts University/USAID, 38.

Statistics. McGraw-Hill Book Co., New York, 258pp.

Economic analysis of the development and usage of thermostable rinderpest vaccine, Proceedings of the Vth International Symposium on Veterinary Epidemiology and Economics. Copenhagen.

Woese C, 1960. Thermal inactivation of animal viruses. Ann NY Acad Sci 83:741-751.



3.1 Buffered Gelatin and Sorbitol

